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## **Sleep-dependent memory consolidation in children**

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## Abbreviations

ANOVA:	Analysis of variance
DLPFC:	Dorsolateral prefrontal cortex
EEG:	Electroencephalography
EOG:	Electrooculography
EMG:	Electromyography
fMRI:	Functional magnetic resonance imaging
LTP:	Long-term potentiation
M1:	Primary motor cortex
MTL:	Mediotemporal lobe
NonREM:	Non rapid eye movement
PFC:	Prefrontal cortex
REM:	Rapid eye movement
SEM:	Standard error of means
SRTT:	Serial reactiontime task
SWA:	Slow wave activity
SWS:	Slow wave sleep
VLPCFC:	Ventrolateral prefrontal cortex

## Introduction

Memories are one of the most fascinating and extraordinary features of human beings. From the cradle to the grave we accumulate great amounts of memories in our brain that enables us to function but also define who we are. The developing brain with its high capacity for plasticity has to acquire basic motor skills like running, writing or speaking as well as fundamental knowledge about how the world is organized. Extensive evidence from studies in adults indicates that sleep after learning new material supports its consolidation in memory, thereby resulting in a stable and long-lasting memory trace (Diekelmann & Born, 2010; Stickgold, 2005; Peigneux, Laureys, Delbeuck, & Maquet, 2001). Children do not only have to learn much but also sleep longer and more intense than adults (Campbell & Feinberg, 2009; Ohayon, Carskadon, Guilleminault, & Vitiello, 2004). Importantly, children display a great amount of slow wave sleep (SWS) which in adults is causally related to the consolidation of memories (Marshall, Helgadottir, Mölle, & Born, 2006; Rasch, Büchel, Gais, & Born, 2007). Thus, children with their immense plasticity in the brain as well as the specific sleep architecture can serve as a model to investigate the fundamental principles and mechanisms of sleep-dependent memory consolidation. In the present thesis, I studied processes of memory consolidation during sleep in children and compared them to adults in three different experiments. Because a recent study reported that sleep did not enhance motor skills in children which is clearly in contrast to the results obtained in adults (Fischer, Wilhelm, & Born, 2007) one major focus was to investigate processes of motor memory consolidation in order to elucidate the specific factors that could explain the lacking effect of sleep on motor memory consolidation.

Before presenting the experimental work in detail, I will give a short overview on the basic principles of memory consolidation, especially on motor memories and introduce the current base of knowledge on the development of memory systems. Subsequently, I will review the present empirical data on sleep-dependent processes of memory consolidation in adults and children to finally deduce the aims and the objectives of the experimental studies. .

## Memory

### *Memory systems and associated brain regions*

In the 1950s the case of patient H.M. taught us a great deal about the existence of different memory systems and their neurophysiological correlates. H.M.'s medial temporal lobe (including the amygdala and bilateral hippocampus) were removed because he was suffering from epileptic seizures. After his recovery from the surgery the epileptic seizures were milder, his short-term memory as well as his IQ remained a little above average but he was not able to build new memories anymore, a phenomenon called retrograde amnesia (Scoville & Milner, 1957; Corkin, Amaral, Gonzalez, Johnson, & Hyman, 1997). More detailed testing revealed that H.M. was able to perform on the mirror tracing task (where he had to trace with a stylo the outer lines of drawn figures that he could only see through a mirror) and to remarkably improve his performance as a consequence of repeated training sessions but he did not remember to ever have done the task before. Together with additional test results it became clear that he was generally unable to store new facts, information and personal experiences (Milner, 1972). These observation as well as those of other brain-damaged patients led researchers to assume the existence of two different kinds of memory systems. The declarative memory system refers to memories for facts (i.e., semantic memory) and autobiographical episodes (i.e., episodic memory) and critically depends on hippocampal functioning. The non-declarative memory system encompasses diverse learning and memory abilities including procedural memories (i.e., memory for skills and habits), priming, conditioning and habituation all of which not necessarily rely on the hippocampus (Cohen & Squire, 1980; Squire, Knowlton, & Musen, 1993; Tulving & Madigan, 1970). According to this theory, declarative memories can be consciously retrieved (i.e., these memories are explicit) whereas procedural memories do not necessarily rely on conscious learning and retrieval capacities (which is referred to as being implicit).

Nowadays, a growing number of evidence challenges a clear theoretical distinction between both memory systems on the basis of hippocampal involvement and consciousness. In a recent review article, Henke mentioned that the two memory systems can be better characterized by the number of trials needed for learning, the complexity of the task and the nature of mental representation (Henke, 2010). In her processing-based theory she postulates that the hippocampus is specifically involved in rapid associative learning with and without consciousness for either long- or short-term storage. This is in line with recent findings

showing hippocampal involvement in motor sequence learning tasks and during associative priming (Curran, Schacter, Norman, & Galluccio, 1997; Eichenbaum, 1999; Schendan, Searl, Melrose, & Stern, 2003; Willingham, Salidis, & Gabrieli, 2002; Albouy et al., 2008). The processing-based theory - like the relational memory theory (Eichenbaum, 1999) - can explain why H.M. was able to perform well on the mirror tracing task but had enormous difficulties to perform on the motor sequence learning task (Susan Corkin, personal communication). In this task, subjects are required to press buttons according to a sequence (Nissen & Bullemer, 1987; Walker et al., 2003a). In order to become faster they need to bind together single elements of a sequence to anticipate the subsequent button according the sequence. This kind of task includes associative learning whereas in the mirror-tracing task a novel visual-response mapping is learned not requiring associative learning. Further evidence for hippocampal involvement in associative memories came from recent imaging studies indicating hippocampus-specific activation whenever a learned item had to be bound together with another item or a context feature whereas the pure item memory activated preferentially the perirhinal cortex (Lehn et al., 2009; Staresina & Davachi, 2009; Tubridy & Davachi, 2010; Dragoi & Buzsaki, 2006; Aggleton & Brown, 2005; Qin et al., 2009). Nevertheless, there is a still open debate about the exact functions of the hippocampus (Henke, 2010; Nadel, Samsonovich, Ryan, & Moscovitch, 2000; Eichenbaum, 2004; Davachi, 2006). Apart from associative learning, the hippocampus was proposed to be specifically implicated in long-term memory storage (Squire & Zola, 1996), in novel tasks (Kumaran & Maguire, 2009) or during spatial learning (Burgess, Maguire, & O'Keefe, 2002; Burgess et al., 2002).

Thus, it became more and more clear that the distinction of memories into two systems, one relying on the hippocampus as well as conscious processing and the other without any hippocampal involvement and consciousness, does not seem to take into account the complex nature of cognitive operations and their neuronal correlates. Accordingly, researchers have proposed new taxonomies and classification schemes but to my knowledge until now none of these can completely explain the variety of phenomena in the field of memory research. With this in mind, it was argued that taxonomies are valuable in inspiring new theories but they cannot serve as theories themselves (Willingham 2001).

### *Stages of memory formation*

Basically, memory consists of three sub-processes, i.e., the encoding of new information, their subsequent consolidation and the retrieval of memories. The original information enters sensory channels and is then encoded into short-term memory. Initially, the new memory representation is highly labile and vulnerable to interfering input. From the great amount of memories that are encoded throughout the day only very few are stored for long-term use (Wilhelm et al., 2011). Offline consolidation results in i) the stabilization of new memory traces (i.e. reduced susceptibility against retroactive interference after retention intervals; Korman et al., 2007; Fischer, Hallschmid, Elsner, & Born, 2002; Brawn, Fenn, Nusbaum, & Margoliash, 2010) as well as in ii) the enhancement of memory performance (McGaugh, 2000; Dudai, 2004; Karni et al., 1998).

On a neurophysiological level, memory consolidation relies on synaptic and system consolidation. Synaptic consolidation is a fast process being completed within several hours after the learning experience (Dudai, 2004; McGaugh, 2000). It involves morphological changes, i.e. the growth of new synaptic connections and the restructuring of existing synaptic connections. In his famous book “The organization of behaviour” Donald Hebb was one the first defining conditions that are required for the occurrence of learning and memory on a synaptic level (Hebb, 1949). He postulated that the functional connectivity between pre- and postsynaptic neurons A and B can change when presynaptic neuron A is repeatedly involved in exciting postsynaptic neuron B. In 1966 Bliss and Lomo were able to uncover the neurophysiological correlates of learning and memory that exactly follow these Hebbian rules (Bliss & Lomo, 1973). A high-frequent stimulation of two neurons was demonstrated to induce a long-lasting enhancement in the signal transmission between the neurons thereby changing the synaptic strength. This phenomenon was called long-term potentiation (LTP). Since then, LTP has been considered a key mechanism behind synaptic plasticity (Cooke & Bliss, 2006; Malenka & Bear, 2004; Kandel, 2001). Nowadays, it has been found that LTP is not per se induced when two neurons fire together but dependent on the exact timing of firing (i.e. spike-time dependent plasticity; Levy & Steward, 1983; Markram, Lubke, Frotscher, & Sakmann, 1997). Synaptic efficacy is enhanced if a presynaptic neuron is activated immediately before the post-synaptic neurons whereas synaptic efficacy is decreased when the post-synaptic neuron fires before the presynaptic neuron (i.e. long-term depression).

Consolidation at the system level is defined by a reorganization of neuronal networks that are involved in a specific memory process (McClelland, McNaughton, & O'Reilly, 1995; Dudai, 2004; Frankland & Bontempi, 2005). The neuropsychological exploration of patients

like H.M. who suffered from temporally graded retrograde amnesia after hippocampal damage inspired researchers to formulate the system consolidation theory (Frankland et al., 2005). This model proposes a rapid storage of newly encoded information in the hippocampus and the neocortex. During the process of consolidation memory representations are reactivated in these networks which lead to a gradual strengthening of cortico-cortical networks thereby incorporating these memories into the existing network of cortical memories (Talamini, Nieuwenhuis, Takashima, & Jensen, 2008; Gais et al., 2007; Takashima et al., 2006). At the same time, the memory representations become more and more independent of the hippocampus which can explain why H.M. was able to remember remote memories even without his mediotemporal lobe. Memory reactivation which is considered to be the basic mechanism within the system consolidation model was observed in the wake state but is even more prominent during sleep (see a detailed description of processes of memory reactivation during sleep in the latter paragraph “Underlying neurophysiological mechanisms”). The process of system consolidation can be boosted by the presence of an associative schema into which new information can be integrated, as shown in a recent study by Tse and colleagues (Tse et al., 2007). In that study, rats learned new associations between the odour of a food and places either with or without a pre-existing schema of the spatial arrangement of food-place associations. Hippocampal lesioning 24 hours after training deteriorated memory performance in the rats without a schema but did not affect performance in the group of rats with an existing schema. Accordingly, the authors concluded that newly encoded pair associations were rapidly incorporated to extrahippocampal (possibly neocortical) areas when a schema already existed.

After successful encoding and consolidation memories are available for retrieval. Several endogenous and exogenous factors have been implicated in the process of memory retrieval. Memory retrieval is enhanced when it occurs in the same context or the same state (e.g. mood) as the encoding of these memories (i.e. context- or state-dependent memory; Godden & Baddeley, 1975; Eich, Stillman, Weingartner, & Gillin, 1975), or when retrieval cues are available (Tulving & Osler, 1968). On the other hand, impairing factors like psychological and physiological stress are capable of preventing the access to stored memories (de Quervain, Roozendaal, Nitsch, McGaugh, & Hock, 2000; Roozendaal, McEwen, & Chattarji, 2009; Kuhlmann & Wolf, 2006).

### *Motor memory*

Motor skills basically belong to the category of non-declarative, procedural memories (Squire & Zola, 1996; Cohen & Squire, 1980). They form a major part of everyday life activities like playing a music instrument, driving a car, writing, dancing or even cooking. Motor skills are acquired by repeated practice and are typically not forgotten once they are completely learned (Song, 2009). In contrast, declarative memories can be learned after one learning trial and are highly susceptible to forgetting (Ebbinghaus, 1885).

The progress of motor learning is reflected by a fast and a slow learning process (Karni et al., 1995; Doyon et al., 2002). The fast learning process manifests itself in profound gains in motor performance occurring within one training session. Performance also improves between two sessions which has been attributed to an ongoing experience-driven slow consolidation process (Karni et al., 1995; Fischer et al., 2002; Walker, Brakefield, Hobson, & Stickgold, 2003a). The progress of motor learning at the behavioral level coincides with multiple changes in the activation of several brain regions. Continuous decreases of activity in the cerebellum, dorsolateral prefrontal cortex (DLPFC) and the parietal cortex was accompanied by increasing activation in the striatum, primary motor cortex (M1) and supplementary motor areas over time (Schendan et al., 2003; Friston, Frith, Passingham, Liddle, & Frackowiak, 1992; Doyon & Benali, 2005; Grafton, 1994; Doyon et al., 2002). Accordingly, it was argued that the early stages of learning recruit mainly cerebello-cortical networks whereas later stages as well as processes of memory consolidation (i.e., between two sessions) seem to rely to a greater extent on striato-cortical areas (Doyon et al., 2005; Debas et al., 2010; Albouy et al., 2008; Bischoff-Grethe, Goedert, Willingham, & Grafton, 2004; Penhune & Doyon, 2002). However, hippocampal activation during acquisition (i.e. the fast-learning process) preceded the offline gain in motor performance (i.e. the slow-learning process) indicating that the fast- and slow learning process are not independent from each other (Albouy et al., 2008; Rauchs et al., 2011).

As described above, procedural memories were originally thought to be completely independent of any consciousness (Squire et al., 1996; Cohen et al., 1980). More recent studies clearly indicate that motor sequences can be learned with and without explicit knowledge (i.e. consciousness) of the sequence structure (Willingham et al., 2002; Schendan et al., 2003; Aizenstein et al., 2004; Fletcher et al., 2005). Explicit and implicit motor learning can be separately scrutinized using the serial reaction time task (SRTT) in which subjects are required to repeatedly press buttons according to an underlying sequence (Nissen et al., 1987). Under implicit conditions, where subjects are unaware of the sequence, they nevertheless

show a significant reduction in reaction times over time for the learned sequence. An explicit mode of learning can be induced by informing subjects about the sequence or instructing them to actively search for the sequence. Explicit and implicit learning processes involve differing but also a number of overlapping brain structures. The hippocampus, the striatum, the parietal cortex, the anterior cingulate and prefrontal regions (e.g., DLPFC) are activated during both explicit and implicit task variations, with a greater activation in the DLPFC during explicit conditions (Schendan et al., 2003; Willingham et al., 2002). Aizenstein et al. (2004) found a clear dissociation of brain activation between explicit and implicit learning of a motor sequence only in the visual cortical areas (Aizenstein et al., 2004). These findings were taken to argue that some types of implicit learning take place even when learning is explicit and that some types of explicit learning occur even during implicit learning conditions (Willingham, 1998).

Thus, explicit and implicit aspects probably operate in parallel in every motor memory task (Shanks & Johnstone, 1999; Willingham, 1998; Ashe, Lungu, Basford, & Lu, 2006) although the exact contribution of these aspects might differ between tasks. The mirror-tracing task or the probabilistic SRTT (in which subjects are required to react as fast as possible on stimuli following an underlying sequence in a probabilistic manner) might include explicit task aspects to a smaller extent than the deterministic SRTT (in which the stimuli follow the underlying sequence in a deterministic manner) or the finger sequence tapping task. Nevertheless, implicit and explicit components are differentially pronounced at distinct stages during the acquisition of a motor skill. Implicit task knowledge has been shown to be available at an early stage of learning and explicit knowledge typically lags behind, providing evidence for a bottom-up direction of motor learning (i.e., learning implicit before explicit knowledge). This delay indicates that explicit knowledge can be extracted from implicit knowledge (Cleeremans, 2008; Sun, Zhang, Slusarz, & Mathews, 2007). Post-learning periods of sleep, and particularly slow wave sleep (SWS), have been reported to crucially support the generation of explicit knowledge on a cognitive task (Wagner, Gais, Haider, Verleger, & Born, 2004; Yordanova et al., 2008; Yordanova, Kolev, Wagner, & Verleger, 2010). On a neuronal level, the emergence of awareness is preceded by activation in the ventral striatum, the right ventrolateral prefrontal cortex (VLPFC; (Rose, Haider, & Büchel, 2010)) and the DLPFC (Willingham et al., 2002). Conceptually, most of the presented literature on the contribution of explicit and implicit aspects in motor learning can be integrated into a recent theory formulated by Cleeremans (2008). The “Radical Plasticity Thesis” postulates that consciousness of a mental representation takes time to develop,

thereby crucially depending on the quality of the representation which is defined by its strength, stability and distinctiveness. According to this theory three stages can be distinguished during motor learning. Processing of new information starts at the point of implicit cognitions. This stage is characterized by weak and poor-quality representations and one cannot exert volitional control over them. In a second step explicit representations emerge, thereby enabling the control over them. The last stage of learning refers to the automatic representation whereby the representation becomes so strong that it does no longer have to be explicitly controlled by the individual. During this stage conscious processing is possible when corrections are required but this does not necessarily happen because the memory trace at this stage has been proven to be adaptive (Cleeremans, 2008).

Whether explicit knowledge helps or hampers implicit task performance depends on several prerequisites. Explicit knowledge deteriorates implicit task performance i) at the earlier stages of motor learning or ii) when a task is difficult or iii) when cognitive resources are less available (e.g., in elderly people). A neurophysiological correlate is provided by the prefronto-hippocampus-dependent system implicated in the emergence of explicit knowledge and cortico-striatal systems underlying implicit skill performance that competitively interact (Willingham, 1998; Poldrack et al., 2001; Albouy et al., 2008). The competitive interaction between the two task aspects was found during acquisition (Fletcher et al., 2005; Poldrack et al., 2001; Albouy et al., 2008; Stefaniak, Willems, Adam, & Meulemans, 2008; Fletcher et al., 2005) but it can extend to processes of memory consolidation (Brown & Robertson, 2007a; Brown & Robertson, 2007b; Robertson, 2009) and even to retrieval (Wagner et al., 2004; Fischer, Drosopoulos, Tsen, & Born, 2006). In contrast, in a well-learned motor task explicit knowledge can result in faster response times because subjects are able to consciously anticipate the location of the successive targets (Willingham, Nissen, & Bullemer, 1989). Moreover, the emergence of explicit knowledge from implicit knowledge is a fundamental component in the progress of procedural learning because only when explicit representations have been built subjects are able to adapt their knowledge to situations that are different from those at learning (Dienes & Perner, 1999; Seger, 1994).

#### *Development of memory systems and neural organization of memories in children*

The human brain undergoes profound changes during development both in its structural architecture and its functional organization (Figure 1; Gogtay et al., 2006; Giedd et al., 1999; Sowell, Trauner, Gamst, & Jernigan, 2002; Casey, Tottenham, Liston, & Durston, 2005). The

number of synapses and neurons rapidly increases shortly after birth (i.e., “synaptogenesis”). From the great amount of synapses only a small part survives and becomes strengthened and fine-tuned by ongoing myelination of axons in order to reach higher level of specificity and to enhance cognitive and neural processes. Whether a synapse survives or not is environmentally regulated thereby crucially depending on the degree of utilization of this synapse. Synaptic pruning (i.e., the loss of synapses) is reflected by a loss of grey matter volume which can be visualized by structural magnetic resonance imaging. Data from several cross-sectional and longitudinal functional magnetic resonance imaging (fMRI) studies indicate a highly specific pattern of maturation in the different brain regions with motor and sensory systems maturing earliest, temporal and parietal association cortices maturing next and prefrontal and lateral temporal regions maturing latest (Casey et al., 2005). The DLPFC seems to mature slowest among prefrontal regions. On the background of these data, Li and colleagues (2006) postulated in the differentiation-dedifferentiation-hypothesis that functional organization of cognitive processes is rather undifferentiated during childhood. It undergoes differentiation resulting in greater specificity that remains largely invariant during adulthood and becomes dedifferentiated again during aging (Li 2006). This hypothesis was supported by recent studies using network analysis of functional connectivity. The child’s brain was less hierarchically organized, possibly in order to allow a higher flexibility in network configuration (Supekar, Musen, & Menon, 2009). Moreover, subcortical areas were more strongly connected in children, whereas adults showed greater connectivity between cortical networks (Supekar et al., 2009).

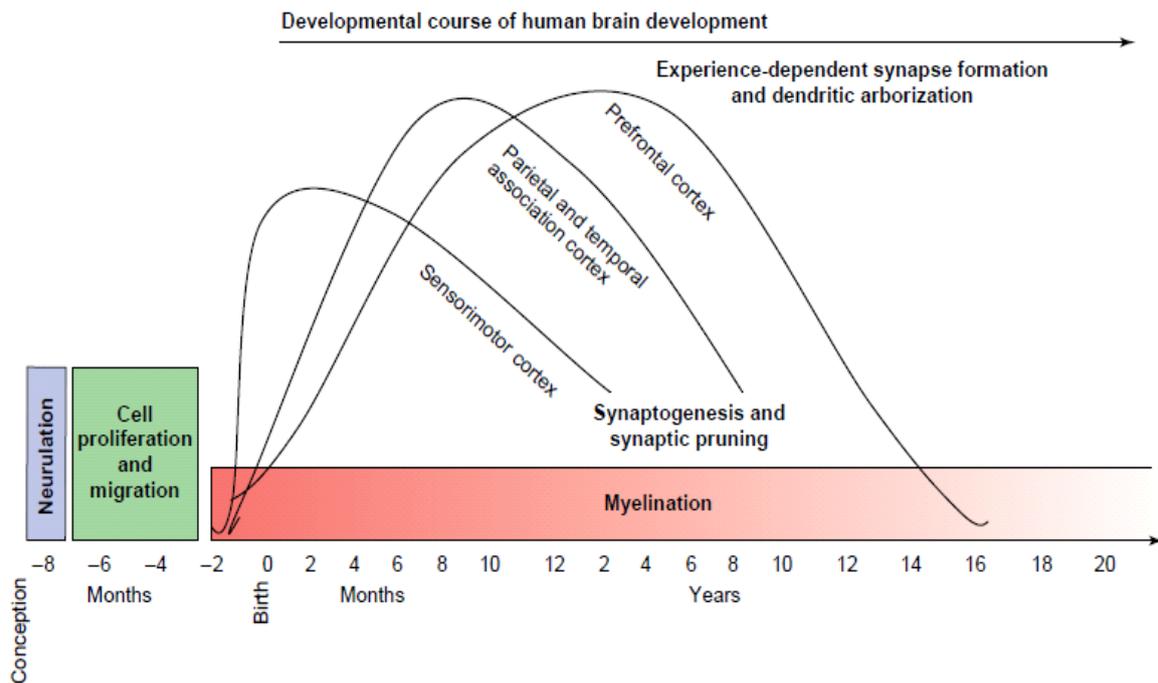


Figure 1. Structural architecture of the developing brain. Developmental changes in the brain including proliferation, migration, myelination and regional changes in synaptic density (Casey et al., 2005).

Structural changes in the organization of the brain coincide with multiple cognitive changes during development. It has been commonly assumed that memory systems follow a hierarchical development, with the non-declarative memory system being adult-like very early in life (i.e., at 3 years of age) whereas the declarative memory system proceeds maturation until early adulthood (Nelson, 1995; Tulving & Schacter, 1990; Naito, 1990). The creation of appropriate measures of memory performance in young children is a great challenge in the research field since basic cognitive abilities like vision, perception, decision making and speaking are necessary in most tasks. Some of the non-declarative memory tasks that have been studied in children among many others are priming for common objects or faces, word-stem priming (Murphy, McKone, & Slee, 2003; DiGiulio, Seidenberg, O'Leary, & Raz, 1994; Drummey & Newcombe, 1995; Hayes & Hennessy, 1996; Gulya et al., 2002; Rovee-Collier, 1997) and the motor sequence learning task (Thomas & Nelson, 2001; Thomas et al., 2004). In very young children when language is not yet fully developed eye movement behaviour either visually scored or with an eye tracker can be used to indicate whether the infant remembers an item or not (Hupbach, Gomez, Bootzin, & Nadel, 2009; Gomez, Bootzin, & Nadel, 2006; Rovee-Collier, 1997).

On a neurophysiological level, the declarative memory system was argued to be developed late because of its reliance on the late-maturing hippocampus and neocortex whereas non-declarative memories are available early in life because it recruits subcortical regions like the basal ganglia and the cerebellum that basically mature earlier (Casey et al., 2005; Bachevalier, 1990). Nevertheless, a great number of studies indicates that the picture is much more complex, thereby challenging the concept of a general developmental dissociation of memory systems (Rovee-Collier, 1997). Murphy and colleagues (2003) argued that four different factors contribute to the dissociation of memory systems, namely knowledge base, basic capacity, memory strategy and meta-memory (Murphy et al., 2003). All factors increase with age due to their reliance on the late-maturing prefrontal cortex (PFC) but basic capacity, memory strategy and meta-memory are typically involved in declarative rather than non-declarative memories. Accordingly, it has been hypothesized that non-declarative memories would also increase with age in a task with an underlying knowledge base that undergoes developmental changes. Murphy and colleagues were indeed able to find profound age-related differences in a priming task relying on category knowledge (Murphy et al., 2003). Moreover, age-related differences were also reported in another domain of the non-declarative memory system, i.e., in motor sequence learning (Fletcher, Maybery, & Bennett, 2000; Maybery, Taylor, & O'Brian-Malone, 1995). Age-dependent differences in a deterministic SRTT were found to be attributed to deficits in explicit task knowledge rather than implicit performance (Thomas et al., 2001) indicating that the greater explicitness of a task the greater the age-related differences that can be found.

Despite the relative wealth of knowledge about the development of memory abilities, little is known about functional correlates of declarative and non-declarative memories in the brain. In a recent study by Ofen et al. (2007) children and adults (4-24 years) learned pictures with in- and outdoor scenes in the fMRI. As expected, greater PFC and mediotemporal lobe (MTL) activation was observed for remembered in comparison to forgotten scenes independent of age. The activation in the PFC but not the MTL covaried with age and the activity in specific PFC regions (i.e., the DLPFC but not the VLPFC) correlated with the age-dependent increase in recognition memory (i.e. the ability to decide in the presence of a stimulus whether this stimulus was previously presented or not). The authors argued that these results reflect faster developmental trajectories for MTL than for specific PFC functions (Ofen et al., 2007). Nevertheless, profound structural and functional changes until adolescence have been reported for MTL regions as well. Despite relative stability of the hippocampal overall size between 4 to 25 years, the volume of the anterior hippocampus decreases over time, whereas

that of the posterior hippocampus increases (Gogtay et al., 2006; Gogtay et al., 2006). During encoding of outdoor scenes hippocampal recruitment successively decreases with age in a group of subjects ranging between 11-19 years whereas the connectivity between the entorhinal cortex and DLPFC increases with age (Menon, Boyett-Anderson, & Reiss, 2005). The authors argued that the greater connectivity between PFC and MTL regions is associated with enhanced effectiveness of encoding strategies and/or a greater awareness of encoding in adults. Furthermore, the MTL seems to be less selective in children compared to adolescents and adults (Ghetti, DeMaster, Yonelinas, & Bunge, 2010). In this study, the subjects' brains were scanned (using fMRI) during the encoding of pictures and were later asked in a recognition test whether they had seen the picture before and if so, in what colour. With this procedure the authors were able to dissociate item and source memory with the later being well-known to be associated with hippocampal functioning (Tubridy et al., 2010). In 14-years-old and adults the hippocampus and posterior parahippocampal gyrus were selectively involved in source memory, whereas in 8-years-old both regions were indiscriminately recruited for source and item recognition (Ghetti 2010). These findings support the notion that the MTL works as a unitary system in children and becomes specialized and increasingly differentiated during development (de Haan 2006).

Until now only one study investigated age-related differences in brain activity during the acquisition of a motor memory task (Thomas et al., 2004). In a SRTT, trials that required the subjects to quickly press buttons according to an underlying 10-elements sequence were alternated with trials including a pseudorandom sequence. Adults clearly outperformed children (7-11 years old) in implicit learning of the sequence (which was indicated by reaction-time differences between sequence and random trials). In parallel, activity in the right hippocampus was greater for sequence trials than for random trials but only in the adults' group. Independent of trial type, adults showed greater activity in cortical regions whereas children showed greater activation of the putamen. Activity in the right caudate nucleus correlated significantly with behavioral measures of implicit learning for both age groups. On the background of these results, the authors suggested that children and adults differentially process a motor learning task, with adults preferentially recruiting cortical areas and children primarily recruiting subcortical areas during task performance.

In sum, the presented data indicate that the assumption of a hierarchical development of memory systems, with the declarative system being adult-like much later than the non-declarative system, can no longer be hold. Instead, task-related factors like the involvement of previous knowledge, strategy use and the contribution of explicit aspects might determine

whether memory performance differs between children and adults. Importantly, all these factors might be mediated by the recruitment of the hippocampus and the PFC.

## **Sleep**

### *General overview (sleep stages, sleep cycles, functions)*

Sleep is a brain state that is characterized by a complete loss of consciousness, by physical quiescence and reduced responsiveness to external stimuli. Circadian and homeostatic factors regulate the occurrence of sleep, with the latter resulting in an increasing sleep pressure during the day and its gradual decrease during sleep (Borbely, 1982; Borbely, 2001). Sleep deprivation induces a profound rebound (in particular of slow wave sleep (SWS) and rapid eye movement (REM) sleep; see below) when sleep is eventually possible. Sleep was often thought to help conserving energy, restoring body functions and avoiding the predators. Recently, the memory function of sleep has received great attention as it might be the only function that possibly explains the complete loss of consciousness because processes of memory consolidation can not occur at the time of acute memory encoding (Diekelmann et al., 2010; Born, Rasch, & Gais, 2006; Marshall & Born, 2007).

Sleep is divided into two types (i.e., REM sleep and NonREM sleep), both of which are characterized by typical electroencephalographic (EEG), electromyographic (EMG) and electrooculographic (EOG) activity. NonREM sleep is further divided into sleep stage 1, 2, 3 and 4 with stage 1 being the lightest and stage 4 being the deepest sleep stage. In a normal human sleep cycle NonREM sleep stages are followed by REM sleep, with each sleep cycle lasting for around 90 minutes. Five to seven sleep cycles occur within one night. Rechtschaffen and Kales (1963) defined standardized criteria to score the different sleep stages in human sleep EEG recordings. Sleep stage 1 defines the transition from wake to sleep state and is accompanied by a reduction of alpha activity (8-12 Hz) within the EEG and slow eye movements. The duration of sleep stage 1 typically does not exceed 5 – 7 minutes. Sleep stage 2 is characterized by the appearance of so-called K-complexes (a positive followed by a negative wave in the sleep EEG), and sleep spindles that are phasic patterns of neuronal activity with a frequency of 10 -15 Hz. Sleep spindles are generated in the thalamus and are thought to induce synaptic plasticity in the neocortex by enhancing cellular  $Ca^{2+}$  influx. The major characteristic of sleep stage 3 and 4, which are summarized as SWS, is the occurrence of slow oscillating brain activity (0.5-4 Hz), which includes delta waves with a frequency of

1-4 Hz and slow oscillations with a frequency of  $< 1$  Hz) with a minimum amplitude of  $75\mu\text{V}$ . REM sleep is defined by a reduction of the muscle tone as well as the occurrence of the characteristic rapid eye movements. However, the EEG during REM sleep shows patterns similar to stage 1 sleep, e.g. theta (5-8 Hz) and alpha-activity (8-13 Hz).

### *Sleep in children and adults*

Sleep changes fundamentally during development with respect to total sleep time, sleep latency, sleep efficiency (i.e., sleep time with reference to time in bed), time awake after sleep onset, and the time spent in the different sleep stages (Figure 2; Ohayon et al., 2004; Grigg-Damberger et al., 2007). In general, total sleep time and sleep efficiency decreases whereas time awake after sleep onset increases during development. The different sleep stages are already distinguishable in the human sleep EEG at 2 months after birth but the absolute and relative amount of time spent in each sleep stage is highly different. Newborns spend almost 50 % of their sleep time in REM sleep which has been suggested to reflect processes of brain maturation (Shaffery, Sinton, Bisette, Roffwarg, & Marks, 2002; Lopez et al., 2008). The percentage of REM sleep rapidly declines to 20%, becoming adult-like at the age of 6 months (Roffwarg, Muzio, & Dement, 1966; Marks, Shaffery, Oksenberg, Speciale, & Roffwarg, 1995). Spindles can be divided into fast centroparietal spindles (i.e. 12-15 Hz) and slow frontal spindles (i.e. 10-12 Hz) with the latter depending to a greater extent on developmental factors (Scholle, Zwacka, & Scholle, 2007). The number of spindles is minimal between 1.7 and 2.3 years of age, highly increases thereafter and reaches a plateau at an age of 5 years which remains up to 16 years. The interspindle interval reaches a maximum at 1.7 and 2.3 years and the amplitude decreases gradually until adolescence (Scholle et al., 2007). Slow wave activity (SWA; 0.5 – 4 Hz) as well as the slow wave amplitude increase until the beginning of puberty at the age of 10-12 years and remarkably decrease thereafter (Jenni & Carskadon, 2004; Ohayon et al., 2004; Campbell et al., 2009; Kurth et al., 2010). Peak levels in SWA at the age of 10-12 years are thought to reflect the high synaptic density and efficacy at a mechanistic level as both factors support processes of neuronal synchronization underlying slow oscillating potentials (Dash, Douglas, Vyazovskiy, Cirelli, & Tononi, 2009). It has been proposed that SWA is a valid indicator of cortical maturation because it is highly correlated with gray matter density and follows the same developmental trajectory as synaptic density and cerebral metabolic rate (Campbell et al., 2009; Buchmann et al., 2010).

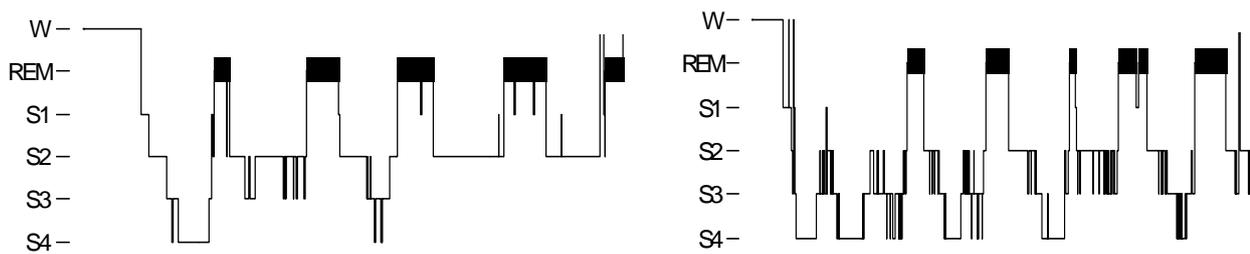


Figure 2. Sleep in children and adults. A typical sequence of sleep stages across the whole night (i.e. hypnogramm) in an adult (left) and a child (right). Sleep stages are w = wake, REM = Rapid Eye Movement Sleep, S1, S2, S3, S4 = sleep stages 1 to 4.

## Memory consolidation during sleep

### *Sleep-dependent consolidation within the different memory systems*

Since almost 100 years it is well-known that sleep benefits processes of memory consolidation (Jenkins & Dallenbach, 1924; Heine, 1914). As a standard design in these studies, subjects learn memory material and are later tested again on the same task after an interval of sleep or wakefulness. Comparing retention performance in those subjects who slept after learning with those who stayed awake during the retention interval generally reveal better performance in the sleep condition (Diekelmann, Wilhelm, & Born, 2009; Stickgold, 2005; Peigneux et al., 2001; Rauchs, Desgranges, Foret, & Eustache, 2005). Sleep-dependent processes of consolidation were reported in a variety of declarative memory tasks, i.e., wordlists with nonsense syllables (Jenkins et al., 1924) or real words (Diekelmann, Born, & Wagner, 2010; Lahl, Wispel, Willigens, & Pietrowsky, 2008; Yaroush, Sullivan, & Ekstrand, 1971), lists with highly, moderately or non-associated word-pairs (Plihal & Born, 1997; Tucker et al., 2006), object-location associations (Diekelmann, Büchel, Born, & Rasch, 2011; Rasch et al., 2007) and neutral or emotional stories or pictures (Groch et al., 2011; Wagner, Gais, & Born, 2001; Wagner, Degirmenci, Drosopoulos, Perras, & Born, 2005). Beneficial effects of sleep were also found for several non-declarative tasks like the finger sequence tapping task (Walker et al., 2003a; Fischer et al., 2002; Korman et al., 2007; Debas et al., 2010), a deterministic or probabilistic SRTT (Fischer et al., 2007; Drosopoulos, Harrer, & Born, 2010; Robertson, Pascual-Leone, & Press, 2004), the visual discrimination task (Gais, Plihal, Wagner, & Born, 2000; Stickgold, James, & Hobson, 2000; Mednick, Nakayama, &

Stickgold, 2003) and the rotary motor task (Huber, Ghilardi, Massimini, & Tononi, 2004). Effects of sleep express themselves in qualitative and quantitative changes of the memory representation (Diekelmann et al., 2010; Marshall et al., 2007). Newly acquired memory representations are strengthened during sleep, which is indicated behaviourally by an enhancement of performance and in a stabilization of memory (i.e., the immunity against interfering test material) at retrieval (Fischer et al., 2002; Korman et al., 2007; Ellenbogen, Hulbert, Stickgold, Dinges, & Thompson-Schill, 2006a; Plihal et al., 1997). Both measures refer to quantitative alterations of the memory trace. Importantly, memories are also qualitatively modified during sleep insofar as they are available at retrieval testing in a form different from that at learning. This has been demonstrated in a study, where subjects performed on a cognitive task (i.e. number reduction task) by using two simple stimulus-response rules (Wagner et al., 2004). Subjects were not informed about the existence of a third abstract rule whose application abruptly improves task performance when subjects become aware of it. More than twice as many subjects gained insight into this hidden rule when they had slept after learning as when they were awake (Wagner et al., 2004; Yordanova et al., 2008). None of the subjects were aware of this rule before sleep indicating that a qualitative modification of memories occurred which is specifically pronounced during sleep. Fischer et al. (2006) confirmed these data by showing that sleep after implicitly learning a SRTT enhances the formation of explicit sequence knowledge (Fischer et al., 2006; Drosopoulos et al., 2010). The extraction of explicit knowledge from an implicitly learned task appeared to be critically related to early night SWA (Yordanova et al., 2010).

A great number of studies has been conducted to investigate the relevance of different sleep stages and specific sleep parameters for consolidation in the two memory systems. Two hypotheses have been formulated in this regard: the sequential hypothesis and the dual process theory (Peigneux et al., 2001; Diekelmann et al., 2010). The dual process theory postulates that the consolidation in both memory systems depends on different sleep stages, i.e., SWS benefiting declarative memories and REM sleep supporting procedural memories. Evidence came from studies using the research paradigm of night-half comparison, which takes advantage of the fact that SWS and REM sleep are unevenly distributed across nocturnal sleep, with SWS dominating the early night and REM sleep dominating the late night. Thus, differential effects of the two sleep stages can be disentangled by comparing processes of consolidation during the early SWS-rich night and the late REM-sleep rich night (Plihal et al., 1997; Plihal & Born, 1999). In the study by Plihal et al (1997), subjects in the early night group learned a task in the evening and were tested after three hours of early SWS-

rich sleep. In the late night group subjects slept 3 hours of SWS-rich sleep before learning the task which was tested after subjects slept for the next 3 hours filled with REM sleep-rich late sleep. In parallel wake control groups, subjects had to stay awake during either the early or late period of the night. Plihal and Born (1997) reported beneficial effects of early SWS-rich sleep for the retention of word-pairs whereas performance on the mirror-tracing task was promoted by REM sleep rich late sleep. However, the clear distinction (i.e., declarative memories during SWS and procedural memories during REM sleep) which is postulated by the dual process hypothesis has been challenged in light of several recent findings. The consolidation of emotional declarative memories is proposed to occur during REM sleep although a contribution of SWS has been confirmed as well (Groch et al., 2011; Wagner et al., 2001; Wagner et al., 2005). Several procedural tasks like the visual discrimination and rotation adaptation also profit from SWS (Stickgold, Whidbee, Schirmer, Patel, & Hobson, 2000; Gais et al., 2000; Huber et al., 2004) whereas the pharmacologically induced suppression of REM sleep does not disturb but rather enhance retention performance in the finger sequence tapping task (Rasch, Pommer, Diekelmann, & Born, 2009).

The sequential hypothesis postulates a sequential processing of memories during the night with early SWS and late REM sleep supporting different sub-processes of consolidation. This hypothesis was originally based on the observation that rats' memory performance is best when a great number of transitions between SWS and REM periods occur during sleep (Langella, Colarieti, Ambrosini, & Giuditta, 1992). Further support for the sequential contribution of both sleep stages came also from studies in humans. Post-sleep performance in the visual discrimination task was correlated both with SWS in the beginning of the night and with REM sleep at the end of the night (Stickgold et al., 2000). Moreover, a nap after learning the visual discrimination task was most beneficial to memory consolidation if sleep contained both SWS and REM sleep (Mednick et al., 2003). Naps containing only SWS prevented the decrease in performance which was seen in the wake condition but there was no performance gain which was seen after sleep including both SWS and REM sleep. Accordingly, the authors assumed that early SWS serves to stabilize memory representations and late REM sleep is functionally related to performance improvement. A sequential processing of memories during sleep has been also proposed by Diekelmann and Born (2010) but these authors assigned different functions to SWS and REM sleep (Figure 3). They argued that during SWS newly acquired memory representations are incorporated into the network of pre-existing memories thereby becoming restructured and reorganized in the process of system consolidation whereas during REM sleep these memory representations become strengthened

in the process of synaptic consolidation (Diekelmann et al., 2010). Further evidence is needed to elucidate the specific functions of SWS and REM sleep for memory consolidation.

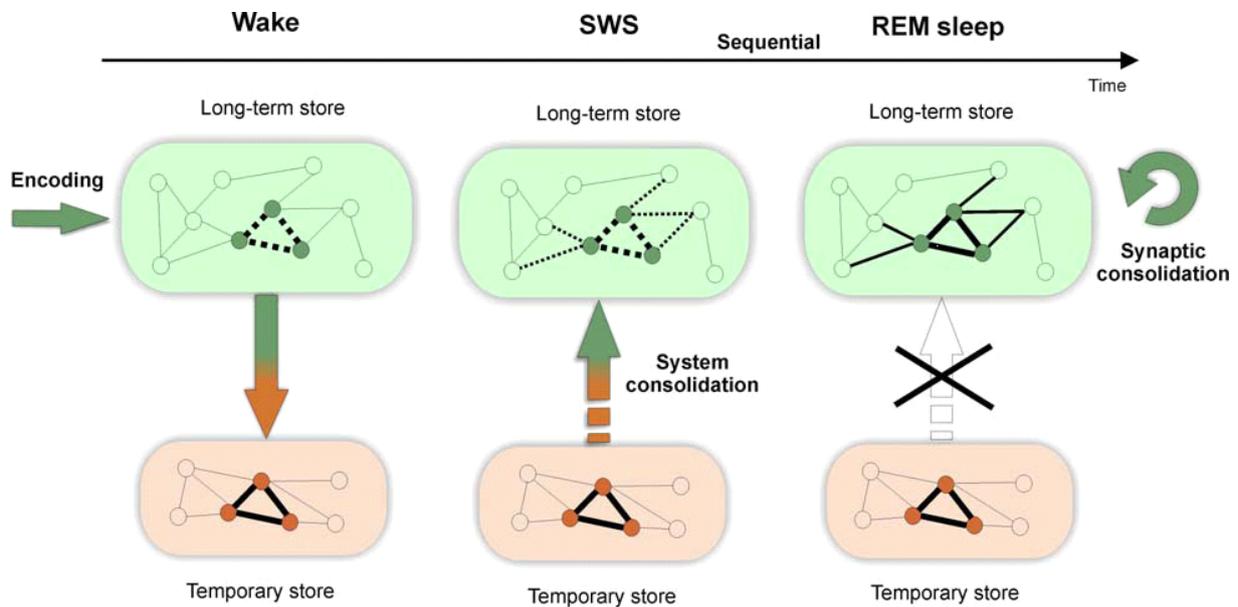


Figure 3. The sequential contribution of REM sleep and SWS to processes of memory consolidation (from Diekelmann and Born, 2010).

### *Modulating factors of sleep-dependent memory consolidation*

From the great amount of information that is encoded throughout the day only a small fraction is consolidated for the long-term. There is ample evidence that a number of factors that are capable of enhancing the future relevance of encoded memories determines whether memories are consolidated during sleep or not (Wilhelm et al., 2011; Payne, Stickgold, Swanberg, & Kensinger, 2008). In a recent study, subjects learned declarative and procedural memory tasks before retention sleep or wakefulness. Half of the subjects were informed about the retrieval test after the retention interval whereas the others were not. Sleep after learning in comparison to wakefulness enhanced memory performance but only in those subjects who expected the retrieval. Retrieval expectancy did not affect memory performance in the wake groups. SWA during the night after learning was enhanced in those subjects who expected the retrieval in contrast to subjects who did not. Moreover, SWA was correlated to memory performance after sleep. Thus, the mere expectation of a future retrieval test determined whether this memory got access to processes of sleep-dependent memory consolidation

(Wilhelm et al., 2011). Motivational factors (i.e., expected monetary reward) as well as the emotionality of learned information can also enhance the individual relevance of memories thereby enhancing processes of sleep-dependent memory consolidation (Fischer & Born, 2009; Payne et al., 2008; Wagner et al., 2001). Although the neurophysiological factors have not been investigated until now, it has been speculated that a prefrontal tagging of those memories that are of future relevance boosts processes of memory consolidation during sleep.

Apart from the relevance of a memory multiple other factors like the performance level at encoding and the explicitness of encoded memories are also capable of influencing sleep-dependent memory consolidation (Diekelmann et al., 2009). Task performance during learning can greatly differ as a function of individual capacities and also of task difficulty. At the beginning of motor learning, performance is usually slow and characterized by a great need for cognitive control. Task performance becomes more and more automated when learning proceeds. These behavioural changes are accompanied by specific neurophysiological patterns (Karni et al., 1998; Willingham, 1998; Doyon et al., 2005). A recent neuroimaging study in adults reported discrete changes in neuronal activation in the course of training of an implicit oculomotor sequence task in subjects with different levels of performance: Slow-learning subjects showed enhanced hippocampal activation until the end of learning whereas in fast learners hippocampal activity decreased and striatal activity increased during training. Interestingly, motor performance was shown to increase after retention intervals containing periods of sleep but only in fast learning subjects with this gain being predicted by hippocampal activation during learning (Albouy et al., 2008). In contrast, Kuriyama (2004) demonstrated sleep-induced performance gains only for those parts of a motor sequence that subjects performed slowly during learning. Further support for the notion that sleep is not beneficial after intense learning came from a study comparing the effects of sleep in subjects with different amounts of training. Subjects that were trained on a motor sequence task in six sessions before sleep did not show any performance improvement after sleep whereas subjects with less training did (Keisler, Ashe, & Willingham, 2007). For declarative memories a similar inconsistent pattern of results has been reported. Investigating effects of sleep on word-lists that were either learned to a criterion of 60 % or 90 % correct responses Drosopoulos and colleagues observed greater benefits of sleep for weakly (i.e., 60 % learning criterion) than for strongly encoded word-pairs (Drosopoulos, Schulze, Fischer, & Born, 2007). The impact of sleep on memory consolidation specifically in those subjects who performed worse in a word list learning task due to high task difficulty was also reflected by greater spindle activity in comparison to subjects with a higher level of task performance. The

number of spindles in low-performers was associated with retention performance in this study (Schmidt et al., 2006). In contrast to these studies Tucker and Fishbein (2008) found beneficial effects of a post-learning nap on retention performance in a word-pair learning task but only in subjects who performed well during encoding. Although contrary at a first glance, these results could be attributed to differences in the tasks that were applied, with the level of familiarity with the task being a possible modulating factor. Performing on an oculomotor sequence task might per se be more complicated than pressing buttons according to an underlying sequence (i.e. in the finger sequence tapping task) making it difficult to compare high- or low performing subjects across studies. Instead, sleep might preferentially benefit performance at an intermediate level which is in line with theoretical assumptions (Stickgold, 2009). However, empirical evidence for this suggestion is still lacking.

There is evidence from studies on procedural memory consolidation that sleep is more efficient when learning involves explicit aspects. Studies using an explicit finger sequence tapping task consistently revealed distinctly greater performance improvements across retention periods filled with sleep compared to wakefulness (Fischer et al., 2002; Walker et al., 2003a; Korman et al., 2007) whereas implicit forms of the SRTT were not shown to benefit from sleep in a number of studies (Song, Howard, Jr., & Howard, 2007). Comparing directly overnight gains in explicit and implicit SRTT performance, two studies revealed significantly larger gains in speed after sleep than wakefulness only when the subjects were aware of the underlying (deterministic) sequence (Robertson et al., 2004; Spencer, Sunm, & Ivry, 2006) whereas gains under implicit conditions were comparable for the sleep and wake control conditions. However, sleep induced significant overnight gains under implicit conditions when subjects were required to respond to contextual cues, i.e. specific coloured stimuli, whose occurrence was correlated with the underlying sequence unknown to the subject (Spencer et al., 2006). The processing of contextual information is known to rely on hippocampal function (Burgess et al., 2002) possibly accounting for the sleep benefits in this context-associated version of the implicit SRTT (Diekelmann et al., 2009).

#### *Potential confounding factors of sleep-dependent memory consolidation*

In spite of the great amount of evidence supporting the beneficial effects of sleep in consolidating declarative and procedural memories, some researchers are still reticent to accept an active role of sleep (Vertes, 2004; Siegel, 2001). Some question sleep's ability to improve task performance whereas others even doubt that sleep passively protects memories

by the reduction of forgetting (Ellenbogen, Payne, & Stickgold, 2006b). A number of confounding factors have been reported in the literature that could also contribute to the improvement of memories after periods of sleep, namely the time of day, fatigue during learning or recall, time to sleep and an averaging of the data (Rickard, Cai, Rieth, Jones, & Ard, 2008; Cai & Rickard, 2009; Rieth, Cai, McDevitt, & Mednick, 2010). More specifically, it was argued that in the evening before sleep the behavioural expression of learning is much lower than the actual learning performance due to circadian factors or increasing fatigue. Learning curves were indeed more flat in the evening than in the morning in an implicit motor learning task (Keisler et al., 2007). Circadian factors as well as time awake since the last sleep period can be controlled for in nap studies in which subjects sleep or stay awake during the same time of day. In a number of studies using this design the beneficial effect of sleep for declarative and procedural memories has been confirmed (Korman et al., 2007; Mednick et al., 2003; Nishida & Walker, 2007). Excluding the influence of fatigue during learning by spaced learning did indeed eliminate the sleep-dependent improvement of performance even though sleep in comparison to wake periods reduced the amount of forgetting (Rickard et al., 2008). Nevertheless, further studies are needed to investigate the role of sleep specifically for motor memory consolidation in different tasks by taking carefully into account all possible confounding factors.

### *Underlying neurophysiological mechanisms*

In one of the first studies on processes of sleep-dependent memory consolidation conducted by Jenkins and Dallenbach (1924), better memory for nonsense syllables after retention sleep than wakefulness was reported. The authors assumed that sleep affects performance by protecting memories from retroactive interference (Jenkins et al., 1924). More specifically, during sleep there is basically no further entry of new information into the brain which - during wakefulness - can disturb the consolidation of formerly encoded memories by interference. Later theories have postulated that sleep does not only passively protect memories from interference but provide unique and optimal properties for the occurrence of memory consolidation (Ellenbogen et al., 2006b; Stickgold, 2005; Born et al., 2006; Marshall et al., 2007).

One of the most prominent theories in this context is the system consolidation theory (Figure 4) which was conceptualized on the basis of the two-stage model of memories (Marr, 1971). In this theory, the role of sleep was specified for processes of memory consolidation

occurring at the system level (Frankland et al., 2005; Diekelmann & Born, 2010). As described above, the model considers the reactivation and redistribution of new memory traces as basic mechanisms of offline consolidation. Therefore, both aspects will be explicitly discussed in the following paragraphs.

Reactivation of newly acquired memories during offline periods has been reported in a number of studies in rats (Pavlidis & Winson, 1989; Wilson & McNaughton, 1994; Ribeiro & Nicolelis, 2004; O'Neill, Pleydell-Bouverie, Dupret, & Csicsvari, 2010). More specifically, neuronal firing patterns that had been present during the exploration of a novel environment were reactivated in the same sequential order in the hippocampus during subsequent SWS. Reactivation of pre-sleep experience-dependent firing patterns was not limited to the hippocampus but was also found in the striatum and the neocortex (Peyrache, Khamassi, Benchenane, Wiener, & Battaglia, 2009; Euston, Tatsuno, & McNaughton, 2007; Pennartz et al., 2004). In humans, signs of reactivation of task-dependent neuronal activity were observed during sleep using fMRI methods (Maquet et al., 2000). Rasch and colleagues were the first to compellingly demonstrate that the reactivation of memories during SWS is causally related to the sleep-dependent enhancement of retention performance by experimentally manipulating processes of reactivation (Rasch et al., 2007). In a series of studies, subjects learned card-pairs in a 2D object location task (similar to the game “concentration”) before retention sleep or wakefulness. During learning subjects were simultaneously presented with an odour which became associated with the task as a context cue. The odour was again presented during subsequent sleep. After the retention interval, subjects were asked for their memory of the card-pairs. Memory performance was superior in those subjects who were re-exposed to the odour during SWS but not during REM sleep or during periods of wakefulness. Re-exposure during SWS also activated the right and left hippocampus indicating that odour presentation indeed boosted the reactivation of memories within the hippocampus (Rasch et al., 2007). The reactivation of spatial memories can be also enhanced using auditory cues during SWS (Rudoy, Voss, Westerberg, & Paller, 2009). Processes of memory reactivation do not only occur during sleep but also during wakefulness (Hoffman and McNaughton, 2002; Karlsson and Frank, 2009). During waking these reactivations lead to a temporary labilization of the memory representation to interfering inputs, which possibly allows for an immediate updating of the memory but requires re-consolidation to re-stabilize the representation (Nader & Hardt, 2009; Sara, 2000). Reactivations occurring during sleep exert an immediate stabilizing effect on memory representations without undergoing labilization (Diekelmann, Büchel, Born, & Rasch, 2011; Rasch et al., 2007).

The system consolidation theory postulates that memories initially stored in the hippocampus are gradually transferred to neocortical sites. Using fMRI, Gais et al. demonstrated that sleep after learning a list of words in comparison to wakefulness led to greater hippocampal activation at a first recall 48 hours after learning (Gais et al., 2007). Sleep also enhanced the functional connectivity between hippocampus and ventromedial PFC – a region well-known to be involved in memory storage (for review see Simons & Spiers, 2003). At a later recall six months after learning, retrieval of the words activated the same prefrontal region to a greater extent in those subjects who had slept after learning compared to those who had stayed awake indicating that sleep induced changes in the representation at the system level.

System consolidation during SWS relies on a hippocampo-neocortical dialogue under control of slow oscillations (~0.75 Hz) that hallmark SWS and are mainly generated in the neocortex. A major function of slow oscillations is to temporally group neuronal activity into global up- and down states (Steriade, Nunez, & Amzica, 1993). Processes of memory reactivation in the hippocampus are indicated by sharp-wave ripple events which temporally coincide with the emergence of thalamo-cortical spindles due to the synchronizing influence of slow oscillations (Ji & Wilson, 2007; Euston et al., 2007). Hippocampal ripples become nested into the single oscillatory troughs of spindles and both reach neocortical networks in the slow oscillating up-state (Siapas & Wilson, 1998; Sirota & Buzsaki, 2005; Mölle & Born, 2009). Spindles are capable to enhance synaptic plastic processes in the neocortex, e.g., by stimulating  $\text{Ca}^{2+}$  influx providing optimal conditions for storing the incoming information. The importance of slow oscillations and spindles for processes of memory consolidation has been confirmed in a number of recent studies (Wilhelm et al., 2011; Gais, Mölle, Helms, & Born, 2002; Mölle, Eschenko, Gais, Sara, & Born, 2009; Marshall et al., 2006). More specifically, spindle activity during the post-learning night or a day-time nap was positively correlated with performance at retrieval in declarative and non-declarative memory tasks (Rasch et al., 2009; Fogel & Smith, 2011; Morin et al., 2008; Gais et al., 2002; Schmidt et al., 2006). Moreover, the number of spindles was increased i) after intense learning of word-pairs, ii) when learned information is expected to be relevant for the future and iii) when a memory task is difficult (Gais et al., 2002; Wilhelm et al., 2011; Schmidt et al., 2006). Moreover, slow oscillations have been reported to be enhanced during sleep after learning with these increases being correlated with the gain in performance after sleep (Clemens, Fabo, & Halasz, 2005; Wilhelm et al., 2011; Huber et al., 2004; Mölle et al., 2009).

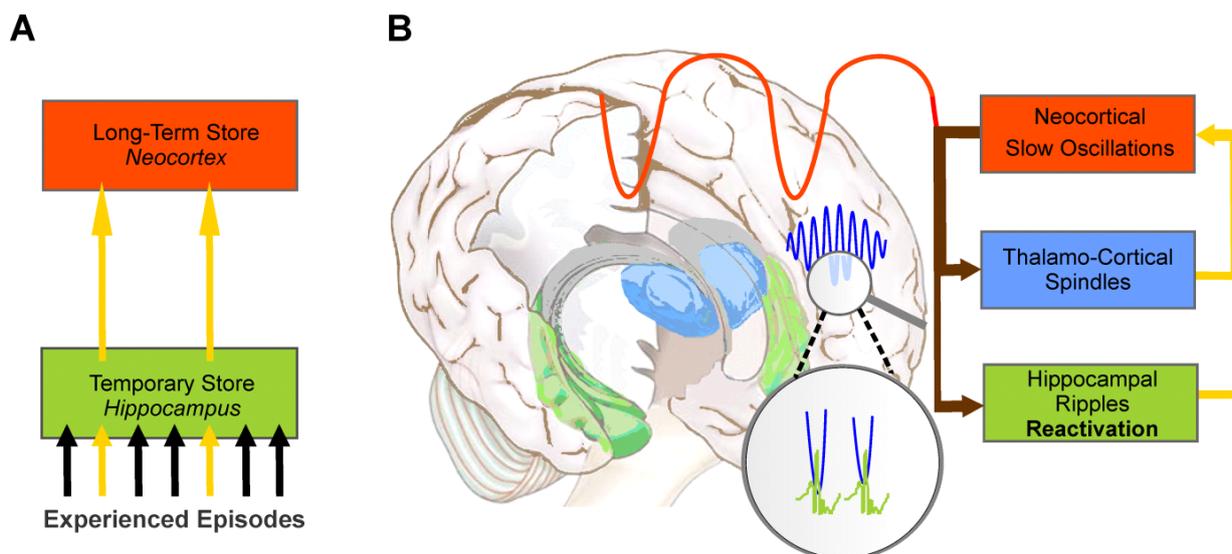


Figure 4. Active system consolidation during sleep. **(A)** Newly acquired memories are encoded into a temporary store (i.e., the hippocampus in the declarative memory system) and become reactivated to be redistributed to the long-term store (i.e., the neocortex) during subsequent periods of slow wave sleep (SWS) **(B)** System consolidation during SWS relies on a dialogue between neocortex and hippocampus under top-down control by the neocortical slow oscillations (red). The depolarizing up-phases of the slow oscillations drive the repeated reactivation of hippocampal memory representations together with sharp-wave ripples (green) in the hippocampus and thalamo-cortical spindles (blue). This synchronous drive allows for the formation of spindle-ripple events where sharp-wave ripples and associated reactivated memory information becomes nested into single troughs of a spindle (shown at larger scale) (from Born and Wilhelm, 2011).

#### *Sleep-dependent memory consolidation in children*

Children with their great plasticity in the brain as well as the specific sleep architecture can serve as a model to investigate the fundamental principles and mechanisms of sleep-dependent memory consolidation. However, memory consolidation during sleep in children has been rarely studied until now. In a first experiment on declarative memory consolidation two conditions were introduced: in the sleep-wake condition, 40 word-pairs were presented in the evening and tested after a first retention interval of sleep in the morning, and again after a second retention interval of subsequent wakefulness (Backhaus, Hoeckesfeld, Born, Hohagen, & Junghanns, 2008). In the wake-sleep condition, children learned the task in the morning and were tested first in the evening and again after retention sleep in the next morning. A significant gain in memory performance was observed after sleep, independent of whether sleep occurred immediately after learning or after a period of wakefulness. Sleep-dependent declarative memory consolidation was positively associated with the amount of NonREM

sleep and negatively related to the amount of REM sleep in both the sleep-wake and wake-sleep condition (Backhaus et al 2008). The fact that the number of recalled word-pairs further increased from the first to the second retrieval (without a further feedback at the first retrieval) after periods of sleep was taken to support the notion of an active role of sleep for declarative memory consolidation in children. However, whether the effects of sleep are even stronger in children than in adults due to the preponderance of SWS remains to be explored in studies using tasks that are highly adapted to the knowledge base in children.

Fischer and colleagues (2007) investigated procedural memory consolidation during sleep in a sample of children and adults. In this study, in children, sleep after training on an SRTT significantly impaired implicit knowledge as assessed by reaction time differences to cue positions that follow the underlying grammar compared to a random sequence (Fischer et al., 2007). Following the wake retention period, implicit performance in the SRTT remained almost unchanged. This pattern clearly differed from that in adults who improved in implicit performance across overnight sleep but showed deteriorated performance after a retention period of wakefulness. This absence of a sleep-dependent gain in motor memories in children is all the more striking as the neuroanatomical structures underlying procedural memory formation mature very early during development (Casey et al., 2005; Gogtay et al., 2006). It was argued that the lack in the sleep-dependent gain in motor performance might be caused by a competitive interaction between explicit and implicit components within a motor task. Due to great amounts of SWS, sleep in children preferentially support the generation of explicit aspects thereby crucially disturbing implicit aspects.

Nevertheless, an immediate lack of overnight gains does not necessarily exclude an improving influence of post-training sleep on the long term: in young zebra-finches learning a song, performance deteriorated across nocturnal sleep when tested directly thereafter. However, the birds that showed strongest post-sleep deterioration achieved a better final song imitation at the end of the 3-month study epoch (Deregnaucourt et al., 2005). Whatever the explanation, these results indicate that procedural memories are differentially processed in children and adults but the mechanisms behind the lacking benefit of sleep on motor learning in children are unclear until now.

As previously mentioned, procedural memory consolidation does not only express itself in an improvement of performance but also in a stabilization of the memory traces. The process of stabilization can experimentally be tested by investigating whether performing on a second motor sequence deteriorates the consolidation of a formerly learnt sequence by retroactive interference. A recent study demonstrated that children were less susceptible to an

interfering sequence after a wake retention interval of two hours than adolescents or adults indicating that processes of motor memory consolidation follows very fast kinetics in children during the wake phase (Dorfberger, di-Japha, & Karni, 2007).

Recent studies in infants also indicate that processes of sleep-dependent memory consolidation during the very early periods of development are different from those in adults. Gomez et al. (Gomez et al., 2006) familiarized 15-month old infants with auditory strings of words of an artificial language in a learning phase. The infant's orienting response, i.e. turning his/her head towards familiar and unfamiliar strings, was used to assess delayed retrieval. Compared to a non-napping control group, children who had napped after learning appeared to be more able to abstract a rule-like pattern underlying the strings of words. However, signs of correct remembering of the presented words were enhanced in the wake group (Gomez et al., 2006). The authors were able to replicate their results in a second study with the same task and the same study design but with a retention interval of 24 hours (Hupbach).

Taken together, the previously mentioned studies indicate an effect of sleep in the consolidation of declarative memories (i.e. word-pairs) in children comparable to adults whereas sleep-dependent gains were not found in a procedural task. Although it was argued by Fischer and colleagues (2007) that this lack might be due to a competitive interaction between explicit and implicit components of a motor task, empirical studies need to be done elucidating the underlying mechanism of a differential effect of sleep on memory consolidation in children and adults.

## **Objectives and hypotheses**

The high capacity for brain's plasticity in children coinciding with much longer and deeper sleep indicate that both factors might be functionally related. First data demonstrated that sleep in children like in adults strengthens declarative memories (Backhaus et al., 2007) but contrary to adults, sleep in children does not support overnight gains of procedural skills (Fischer et al., 2007). In the present thesis the aim was to further explore sleep's role for memory consolidation in children thereby elucidating the factors that could explain the lacking effect of sleep for the consolidation of motor memories. Three studies were performed for this purpose.

Study 1 aimed to investigate sleep's role for declarative and procedural memory consolidation in children by using tasks that are highly adapted to the knowledge base of 6-8

years old children. With respect to declarative memories, children performed on a task that is known to be relatively easy and interesting for them, i.e. the game concentration. It was further aimed to replicate the findings on lacking effects of sleep on motor memory consolidation which was reported by Fischer et al. (2007) thereby also applying a less complex motor memory task (i.e., finger sequence tapping task). In the finger sequence tapping task subjects are required to learn a 5 – elements sequence by repeatedly pressing buttons according to this sequence which might be much easier for children than learning a probabilistic sequence where sequence trials were alternated by random trials. In this first study we formulated the following hypothesis: 1) Sleep benefits declarative memories to a greater extent in children than adults due to the preponderance of slow wave sleep (SWS); 2) SWS is positively correlated with retention performance in the declarative memory tasks; and 3) sleep benefits the offline gain in motor performance in adults but not in children.

As described above, explicit (i.e. declarative) and implicit (i.e. procedural) processes operate in parallel during motor learning (Willingham, 1998; Shanks et al., 1999). It was argued that in children sleep preferentially benefits explicit task aspects within a motor task due to great amounts of SWS which competitively interacts with implicit task performance (Fischer et al., 2007). In Study 2 it was therefore tested whether sleep benefits the extraction of explicit knowledge from an implicitly learned motor task in children and adults. It was hypothesized that 1) sleep benefits explicit task knowledge in both age-groups but 2) to an even greater extent in children which is 3) associated to the high amounts of SWS in this age-group. In this study, the neurophysiological correlates of the extraction of explicit knowledge in children and adults were additionally explored by using fMRI. On the background of recent studies indicating that memories are reactivated in the hippocampus during slow wave sleep thereby possibly strengthening hippocampus-dependent task aspects it was hypothesized that the superior explicit knowledge in children also coincides with 4) greater hippocampal activation during sequence retrieval after retention sleep.

The performance level at learning modulates processes of sleep-dependent memory consolidation in adults (Diekelmann et al., 2009; Albouy et al., 2008; Kuriyama, Stickgold, & Walker, 2004). Stickgold et al. (2009) proposed that sleep preferentially benefits memories at intermediate levels but neither for strong nor weak memory traces (Stickgold, 2009). Children's motor performance is much slower and less automated than adults' performance (Thomas et al., 2004; Fischer et al., 2007; Dorfberger et al., 2007) which might explain why motor memories did not benefit from periods of sleep in this age-group. The purpose of Study 3 was therefore to experimentally increase performance at learning in children (aged 4-6

years) in order to reach intermediate performance levels by manipulating the amount of training. In a parallel control group of adults, motor performance at the end of learning was slowed down by restricting the amount of training to a minimum. Since the task is very easy we expected the reduction of training in adults possibly resulting in intermediate levels at learning that are slightly comparable to the performance level in the group of children with extended training. It was hypothesized that 1) sleep-dependent motor memory consolidation is obvious in both age-groups at intermediate levels but not in the high- and low-performing groups.

# Study 1 - Sleep-Dependent Consolidation of Declarative and Procedural Memories in Children

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## Introduction

Compelling evidence has been accumulated that sleep supports the consolidation of newly acquired memories in adults (Maquet, 2001; Stickgold, 2005; Born et al., 2006). Memory consolidation during sleep is a process of system consolidation which relies on repeated covert reactivations of the neuronal networks encoding the memory and leads to quantitative as well as qualitative changes in the neuronal representations (Wagner et al., 2004; Dudai, 2004; Orban et al., 2006; Ji et al., 2007; Rasch et al., 2007). Sleep in adults strengthens declarative as well as procedural memories (Plihal et al., 1997; Fischer et al., 2002; Walker et al., 2003a; Ellenbogen et al., 2006a). The declarative (i.e., explicit) memory system is related to episodes and facts, whose encoding and short-term retrieval relies critically on the hippocampus aside from prefrontal regions (Squire et al., 1993). For long-term storage, declarative memory is presumably transferred to neocortical networks and thereby becomes independent of the hippocampus (McClelland et al., 1995). Procedural memory refers to the memory for sensory and motor skills that can be learned implicitly or explicitly but do not essentially require hippocampal function. Storage of motor skills involves primarily cortico-striatal and cerebellar circuitry (Doyon et al., 2005). Apart from contributions of non-rapid eye movement (NonREM) sleep stage 2 (Gais et al., 2002; Fogel & Smith, 2006), declarative memories benefit particularly from slow wave sleep (SWS), whereas procedural memories benefit more from REM sleep (Plihal et al., 1997; Peigneux et al., 2004; Marshall et al., 2007). Declarative memory consolidation during SWS relies critically on neocortical slow oscillations that hallmark this sleep stage and drive the reactivation of hippocampal memories during SWS (Marshall et al., 2006; Ji et al., 2007; Clemens et al., 2007).

Sleep in children contains a distinctly greater amount of SWS compared with sleep in adults (Campbell et al., 2009; Ohayon et al., 2004). In parallel, the early developmental period is characterized by a great extent of brain and behavioural plasticity determining the child's

capability to rapidly acquire huge amounts of facts and to effectively shape skills in response to environmental challenges (Li, Brehmer, Shing, Werkle-Bergner, & Lindenberger, 2006; Brehmer, Li, Muller, von, & Lindenberger, 2007). Restriction of sleep in school-children was shown to be associated to impairments in different cognitive functions (Carskadon, Harvey, & Dement, 1981; Randazzo, Muehlbach, Schweitzer, & Walsh, 1998; Steenari et al., 2003). Animal studies provided considerable evidence that developmental sleep, like sleep in adults, is crucially involved in brain plasticity (Dang-Vu, Desseilles, Peigneux, & Maquet, 2006). However, the role developmental sleep plays for the consolidation of memory has only been scarcely examined.

Considering the great amount of SWS in children together with this sleep stage's beneficial role for declarative memory consolidation evidenced in adults, sleep during development may be expected to particularly enhance consolidation of declarative memory. On the other hand, evidence has been provided that maturation of hippocampal and prefrontal brain circuitry underlying declarative memory function is slow and not complete before adolescence (Casey et al., 2005; Gogtay et al., 2006) whereas procedural memory function matures mainly within the first 3 years of childhood and then is maintained at a fairly constant level independent of age (Chugani, Phelps, & Mazziotta, 1987; Meulemans, van der, & Perruchet, 1998; Thomas et al., 2001). On this background, in young children sleep-dependency might be stronger for procedural than declarative memories. However, contrary to this expectation, in developing birds learning a song, Deregnaucourt et al. (Deregnaucourt et al., 2005) observed a deterioration rather than improvement of song structure after nocturnal sleep. In a recent human study (Fischer et al., 2007), children aged 7-11 years, in contrast to adults, likewise showed impaired rather than improved implicit sequence knowledge in a procedural serial reaction time task (SRTT) when training was followed by periods of sleep, pointing towards differential dynamics of sleep-dependent consolidation of procedural memories during development.

Here we aimed to dissociate effects of post-learning sleep on procedural and declarative types of memories in young children (aged 6-8 years) and adults. Retention across intervals of wakefulness during daytime and nocturnal sleep was examined using a 2D-object location task and a word-pair associate task for declarative memory testing and a finger sequence tapping task for procedural memory testing. All tasks had been proven sensitive to the consolidating effect of sleep in previous studies in adults (Walker et al., 2003a; Gais, Lucas, & Born, 2006; Rasch et al., 2007). We show that children's sleep, like sleep in adults,

facilitates declarative memory consolidation but, contrasting with findings in adults, impairs rather than improves motor skill memory.

## **Methods**

**Participants.** Fifteen healthy children between 6 – 8 years of age (mean  $\pm$  SEM:  $7.5 \pm 0.16$  yr; 9 females, 6 males) and 15 healthy adults ( $26.5 \pm 1.3$  yr; 13 females, 2 males) participated in the study. The subjects were recruited via advertisements placed at the university and local after-school care clubs. Interviews with the parents and children as well as standardized questionnaires ensured that the children had no behavioural problems, cognitive impairments or sleep disorders. Children as well as adults had no history of any neurological or psychiatric disorder and did not take any medication at the time of experiments. We carefully surveyed the children's and adults' sleep schedules in order to adapt bedtimes during the study to the subject's habitual bedtime. All subjects were adapted to polysomnographic recordings during a night preceding the experiments proper. The study was approved by the local ethics committee and subjects gave written informed consent before participating. For the children this was accomplished by a parent. Additionally, all children provided verbal assent.

**Procedure and Design.** Each subject participated in two conditions, a “sleep” and a “wake” condition which were conducted at the subject's home. The subject's two sessions were separated by an interval of at least one week, and the order of conditions was balanced across subjects. In the sleep condition, learning started at ~8:00 PM for the children and at ~10:00 PM for the adults after subjects had been prepared for polysomnographical recordings. The learning period varied between 30 and 60 minutes. Subjects went to bed and lights were turned off at the habitual time for children (between 7.30 and 9.30 PM) and adults (between 10 and 12 PM). In the interval between learning and going to bed, subjects prepared for bedtime, brushed their teeth etc. The next morning subjects were awakened at their usual time. Retrieval testing took place ~60 minutes later. The interval between learning and retrieval testing averaged 11 hrs. In the wake condition learning took place in the morning ~60 min after awakening from nighttime sleep and retrieval was tested after a retention interval of wakefulness that again lasted ~11 hours. During the wake retention interval subjects followed their daily schedules. The parents kept a continuous record of the children's activities in order to exclude possible disturbing influences by extraordinary stress or interfering cognitive activities. Before learning and retrieval testing in both conditions

subjects estimated their subjective tiredness and motivation. Children did so by oral report and adults filled in a standardized questionnaire.

**Memory Testing.** To assess retention of declarative and procedural memories across sleep and wake intervals, three different tasks were applied. Declarative memory was tested using a word-pair associate learning task and a 2D-object location task. Procedural memory was tested by a finger sequence tapping task. In each session, the 2D-object location task was tested first, followed by finger sequence tapping and the word-pair associate learning task. For each of these tasks parallel versions were used for testing on the subject's two conditions (sleep, wake). The order of task versions was balanced across subjects.

The word-pair associate learning task required the children to learn a list of 20 word-pairs. For the adults the list included 40 word-pairs. At learning, the experimenter read out loud all word-pairs of the list at a rate of ~1 word-pair/ 5 sec. Then, she read the first (cue) word of each pair in random order and the subject had to name the associated word. Feedback about correctness was given in each case by the experimenter who re-named the correct response if the subject gave no or an incorrect answer. The cued recall procedure was repeated until the subject reached a criterion of 60% correct responses. At retrieval testing after the retention interval the same cued recall procedure was used as during learning. The word-pairs were taken from the "Handbuch deutschsprachiger Normen" (Hager & Hasselhorn, 1994) which provides moderately associated word-pairs for different age groups. To fill up the lists, additional word-pairs were constructed, and a pilot study assured that the degree of association for these word-pairs was comparable with those taken from Hasselhorn (1994). Examples of word-pairs from the children's list are (translated from German) "dolphin and seal" and "eye and crab", and from the adults' list "ear and tone" and "shower and lime".

The 2D-object location task resembles the game "concentration" and consists of 15 card-pairs showing colored pictures of different animals and every-day objects. Throughout the task, all 30 possible spatial locations are shown as grey squares on a 15" flat screen ("the back of the cards"). The locations are geometrically ordered in a checkerboard-like fashion. At learning, the first card of each card-pair was presented alone for one second followed by the presentation of both cards for three seconds. After an inter-stimulus interval of three seconds, the next card-pair was presented in the same way. The whole set of card-pairs was presented twice in different order. Immediately after these two runs, recall of the spatial locations was tested using a cued recall procedure, i.e., the first card of each pair was

presented and the subject had to indicate the location of the second card with a computer mouse. Visual feedback was given in each case by presenting the second card at the correct location for two seconds. The cued recall procedure was repeated until the children reached a criterion of 40% correct responses. The criterion in adults was 60 % correct responses. After presenting a card-pair, both cards were replaced by grey squares again, so that the guessing probability remained the same throughout each run. At retrieval testing the next morning, the same cued recall procedure was used as during the learning phase.

The finger sequence tapping task was adopted from (Walker et al., 2003a) with slight modifications to adjust it to the use in young children. It requires the subject to press repeatedly one of two five-element sequences (4-1-3-2-4 and 2-3-1-4-2) on a keyboard with the fingers of his non-dominant hand as fast and as accurately as possible. To keep working memory demands at a minimum, four horizontally arranged boxes (corresponding to the keys) were displayed on a screen in front of the subject, and a white star successively appeared in the box cuing the next key to be pressed. Star presentation in the respective box was triggered by the preceding key press and was ended by the required key press. At learning, subjects performed on twelve 30-s trials each interrupted by 30-s breaks. Retrieval testing included three trials which were performed after subjects had one warming-up trial. To control for non-specific changes in motor performance, at retrieval testing a new sequence (not learned previously) was introduced subsequent to retrieval testing. Performance on three trials of this novel sequence was tested after subjects had one practice trial.

**Sleep Recordings.** Standard polysomnographic recordings were obtained using a portable amplifier (SOMNOscreen EEG 10-20, Somnomedics, Kist, Germany). Recordings were visually scored offline according to the criteria by Rechtschaffen & Kales (Rechtschaffen & Kales, 1968). For each night, sleep onset, total sleep time, and the time as well as the percentage of total sleep time spent in the different sleep stages were determined. Sleep stages are wake, NonREM (REM – rapid eye movement) sleep stages 1, 2, 3, and 4, slow wave sleep (SWS, i.e. the sum of stage 3 and 4 sleep) and REM sleep. Sleep onset latency (i.e., the first occurrence of a period of stage 1 sleep followed by stage 2 sleep) was determined with reference to the time of lights off. Latencies of SWS and REM sleep were determined with reference to sleep onset.

**Data Reduction and Statistical Analyses.** To indicate memory retention on the word-pair associate learning task and on the 2D-object location task, we used the difference in the

number of recalled items at retrieval testing minus the number of recalled items on the criterion trial at learning before the retention interval. Finger sequence tapping performance was assessed with regard to accuracy (number of correct key presses per trial) and speed (number of key presses per trial). Since both measures reveal essentially the same results this report will be restricted to the accuracy measure. Changes in performance across the retention intervals of sleep and wakefulness were determined by the difference in average performance on three retrieval trials minus performance on the last three trials at learning.

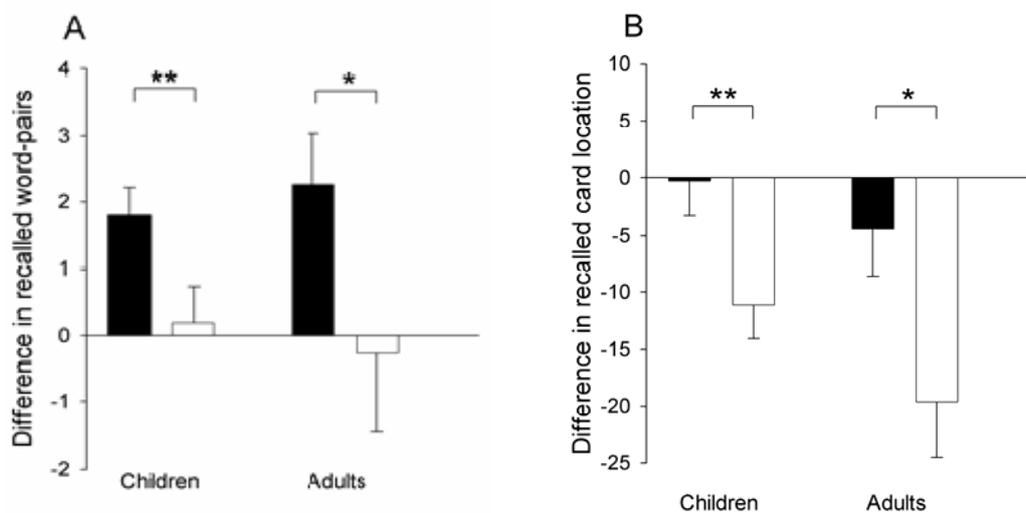
Finger sequence tapping data from one child who did not complete the task were excluded. Polysomnographical recordings from one adult and one child could not be analyzed due to technical failure. Statistical comparisons on measures of memory retention were based on analysis of variance (ANOVA) including the within-subjects factor 'sleep/wake' (representing the sleep and wake conditions) and the between-subjects factor 'age' group (children/adults). Post-hoc comparisons were performed using Student's t-tests. Analyses of performance at learning for the finger sequence tapping task included an additional factor 'trial' representing average performance on the first three and the last three trials of the twelve trials of the learning period. For exploratory purposes, Pearson's correlations were calculated between the time spent in specific sleep stages and changes in memory measures after retention sleep, separately in children and adults.

## Results

**Declarative Memory.** On the word-pair associate learning task, both children and adults remembered more word-pairs after the retention interval filled with sleep than after the wake retention interval ( $F(1, 28) = 17.14$ ;  $p < 0.001$ , for main effect of 'sleep/wake';  $p < 0.001$  and  $< 0.05$ , for pairwise comparisons between sleep and wake conditions in children and adults, respectively; Figure 5A). Retention rates in terms of the absolute as well as the relative difference in performance at retrieval minus performance at learning were comparable in children and adults ( $p > 0.50$ , main effect of 'age', for both comparisons) and so was the enhancing effect of sleep on retention rates ( $p > 0.35$ , 'sleep/wake' x 'age' interaction, for both comparisons). Performance at the criterion trial during the initial learning period was comparable in the sleep and wake conditions for both children (sleep  $14.3 \pm 0.40$  words, wake  $14.3 \pm 0.50$  words) and adults ( $30.9 \pm 1.04$  and  $30.9 \pm 1.41$  words, respectively;  $p > 0.27$ , for all comparisons). Also the number of trials the subjects needed to reach the criterion at

learning did not differ between conditions in children (sleep  $2.3 \pm 0.32$ , wake  $1.9 \pm 0.21$ ) and adults ( $1.3 \pm 0.13$  and  $1.3 \pm 0.12$ , respectively;  $p > 0.2$  for both comparisons).

Retention of card-location on the 2D object location task was also better after the sleep interval than after wakefulness in children as well as adults ( $F(1,28) = 10.77$ ;  $p < 0.01$ ; main effect of ‘sleep/wake’;  $p < 0.01$  and  $< 0.05$ , for pairwise comparisons between sleep and wake conditions in children and adults, respectively; Figure 5B). Performance at learning was closely comparable between sleep and wake conditions in both children and adults. At the criterion trial children recalled in the sleep condition  $7.0 \pm 0.29$  card-locations and in the wake condition  $7.4 \pm 0.31$  locations ( $p > 0.27$ ). The number of trials to criterion was  $2.0 \pm 0.32$  and  $2.1 \pm 0.40$ , respectively ( $p > 0.7$ ). Criterion performance in the adults averaged in the sleep condition  $10.0 \pm 0.31$  card-locations ( $3.1 \pm 0.35$  trials to criterion) and in the wake condition  $10.2 \pm 0.38$  card-locations ( $2.7 \pm 0.44$  trails to criterion;  $p > 0.20$ , for both comparisons).



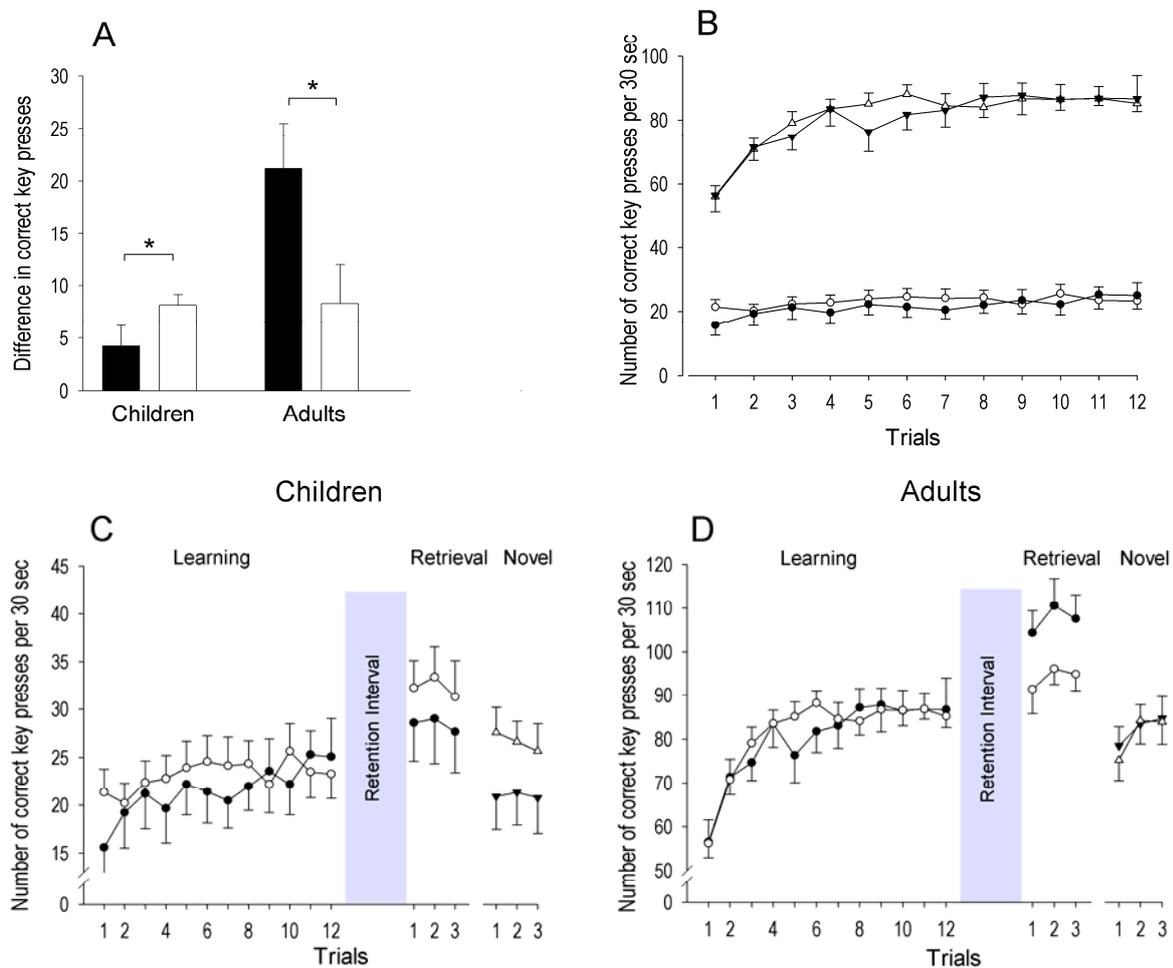
**Figure 5.** Retention performance on both declarative memory tasks in children and adults. Mean ( $\pm$  SEM) retention (**A**) of word-pairs on the word-pair associate learning task and (**B**) of card-locations on the 2D-object location task across intervals of nocturnal sleep (black bars) and daytime wakefulness (empty bars) in children (left) and adults (right). Retention performance is indicated by the absolute difference in the number of recalled items (word-pairs and card-locations, respectively) at retrieval testing after the retention interval minus the number of items recalled at the criterion trial at learning before the retention interval.\*  $p < 0.05$ , \*\*  $p < 0.01$ , for pairwise comparisons between sleep and wake conditions within age groups.

**Procedural Memory.** Changes in finger sequence tapping performance between learning and retrieval testing were in opposite direction in children and adults ( $F(1,27) = 7.77$   $p < 0.01$ ; for

‘sleep/wake’ x ‘age’). In adults, compared with wakefulness, sleep during the retention interval caused the expected gain in motor skill (increase in number of correct key presses trial with reference to learning; sleep:  $21.2 \pm 4.27$ , wake:  $8.3 \pm 3.68$ ,  $p < 0.05$ ). In contrast, children showed better performance after the wake retention interval compared to sleep (sleep:  $4.2 \pm 1.97$ , wake:  $8.1 \pm 1.00$ ,  $p < 0.05$ ; Figure 6A). In children, performance on the novel sequence introduced at retrieval testing was also better in the wake than sleep condition ( $F(1,13) = 4.88$ ;  $p < 0.05$ ; Figure 6C). In the adults performance on this control sequence did not differ between conditions ( $p > 0.65$ ; Figure 6D).

Analysis of the 12 trials of initial training revealed that, as expected, children were generally slower than adults ( $F(1,27) = 197.84$ ,  $p < 0.001$ , main effect of age; Figure 6B). Performance across the training trials clearly improved in both groups ( $F(1,27) = 25.51$ ,  $p < 0.001$ , for main effect of ‘trial’ in a comparison of performance on the first and last three trials,  $p < 0.01$  for separate pairwise comparisons in both children and adults). Importantly, training performance did not differ between the sleep and wake conditions, neither in children nor in adults ( $p > 0.15$ , for all comparisons including tests at single time points).

Based on studies suggesting that delayed gains in skill depend on whether improvement during training had reached saturation (Hauptmann, Reinhart, Brandt, & Karni, 2005), we analyzed saturation by calculating the difference in improvement as estimated by linear regression beta weights across trials 1-4 versus trials 9-12. Thus, a great difference value indicated performance levels close to saturation in the end of training. As expected, adults achieved higher levels of saturation during learning than children ( $F(1,27) = 8.33$ ,  $p < 0.01$ ). Except for a slight positive correlation with performance gains during wakefulness in children ( $r = 0.47$ ,  $p = 0.10$ ), saturation was not associated with retention performance ( $p > 0.24$  for all comparisons).



**Figure 6.** Finger sequence tapping performance in children and adults. Mean ( $\pm$  SEM) values are indicated. **(A)** Gain in finger tapping skill across retention periods of nocturnal sleep (black bars) and daytime wakefulness (empty bars). **(B)** Performance at learning in children (circles) and adults (triangles) before retention intervals of nocturnal sleep (filled symbols, thick lines) and daytime wakefulness (empty symbols, thin lines). **(C)** and **(D)** summarizes finger sequence tapping performance separately in children and adults for the 12 trials at learning before retention intervals of sleep (filled symbols, thick lines) and wakefulness (empty symbols, thin lines) and for the 3 test trials at retrieval thereafter. Following retrieval, performance on a novel sequence was tested which had not been trained at learning.

**Sleep and Reported Tiredness and Mood.** Table 1 summarizes polysomnographic results. Compared with adults, children slept longer ( $t = 6.81$ ;  $p < 0.001$ ), had a lower proportion of lighter sleep NonREM sleep stage 2 ( $t = -3.43$ ;  $p < 0.01$ ) and a greater proportion of SWS ( $t = 2.89$ ;  $p < 0.01$ ). Children showed a shorter SWS latency ( $t = -4.4$ ;  $p < 0.001$ ) and a longer REM sleep latency ( $t = 8.41$ ;  $p < 0.001$ ) than adults. None of the sleep parameters was significantly associated with the gain in memory performance for any of the three tasks in

both age groups ( $r < 0.32$ ). Subjective feelings of tiredness and motivation rated before learning and before retrieval did not indicate any differences between the sleep and the wake condition in adults or children.

**Table 1. Sleep during nocturnal retention interval**

	Children	Adults
Total sleep time, min	567.05 ± 16.59 **	413.15 ± 17.93
Sleep onset, min	17.25 ± 3.81 <sup>(1)</sup>	22.38 ± 5.76
SWS latency, min	8.65 ± 0.82 **	18.04 ± 1.94
REM latency, min	174.60 ± 5.92**	80.96 ± 9.35
<b>Sleep stages - time in minutes</b>		
Wake time	12.55 ± 8.41	5.54 ± 1.38
Stage 1 sleep	14.80 ± 3.90	13.54 ± 2.88
Stage 2 sleep	239.75 ± 17.51	215.54 ± 11.92
SWS	170.60 ± 22.36 **	82.61 ± 12.19
REM sleep	128.15 ± 7.06 **	94.69 ± 9
<b>Sleep stages - time in percent of total sleep time</b>		
Wake time	2.35 ± 1.63	1.33 ± 0.33
Stage 1 sleep	2.69 ± 0.78	3.23 ± 0.61
Stage 2 sleep	42.31 ± 2.67 **	52.46 ± 2.26
SWS	29.92 ± 3.73 *	20.01 ± 2.44
REM sleep	22.52 ± 0.90	22.65 ± 1.59

Mean (± SEM) total sleep time, time to sleep onset (after lights off), latency of first period of SWS and REM sleep (with reference to sleep onset), and time spent awake, in stage 1 sleep, stage 2 sleep, SWS (i.e., sum of stage 3 and 4) and REM sleep given in minutes and percentage of total sleep time. Asterisks indicate significant difference in children in comparison with adults. \*  $p < 0.05$ , \*\*  $p < 0.01$ . <sup>(1)</sup> Sleep onset time in children is biased because they were allowed to follow habitual routines (like listening to a song) after turning off lights.

## Discussion

Our results in adults replicate previous findings showing that retention of declarative and procedural memories benefit from a period of sleep in comparison with wakefulness after learning (Walker et al., 2003a; Gais & Born, 2004; Walker, 2005; Gais et al., 2006; Robertson et al., 2004; Ellenbogen et al., 2006a). By contrast, in the children the effect of sleep on retention depends on the type of memory task: Like in adults, retention of declarative memories was distinctly enhanced when sleep followed learning. However, finger sequence tapping improved more over daytime wakefulness than nighttime sleep. Sleep in children was as expected characterized by greater amounts of SWS and less time spent in NonREM sleep

stage 2. Selective gains in finger tapping skill during wakefulness in children contrasting with sleep-dependent gains of skill in adults, speak for a differential role of sleep for the ‘offline’ consolidation of procedural memories during development.

Influences of sleep on indicators of memory consolidation have been barely examined in children (Gomez et al., 2006; Fischer et al., 2007). Our data provide novel evidence that the supportive effect of sleep on declarative memory consolidation observed in adults is likewise present in young, 6-8 years old children. Consolidation of hippocampus-dependent memories benefits in particular from SWS (Plihal et al., 1997; Marshall et al., 2006). The underlying mechanism involves reactivation of newly acquired memories in hippocampal networks that is time-locked to neocortical slow oscillations during SWS, and presumably facilitates the spreading of respective declarative memory representations to neocortical networks (McClelland et al., 1995; Marshall et al., 2007). Considering the distinctly higher amount of SWS in children than adults, declarative memory consolidation might be expected to benefit from sleep to an even greater extent than in adults. However, overnight retention in terms of absolute differences in recalled card-locations and word-pairs, respectively, with reference to learning before sleep did not differ between children and adults, and there was also no difference between the age groups in retention if expressed as percentage of items recalled at learning. However, quantitative comparisons between the groups are hampered because of clear differences in learning between the groups. Children learned the word-pairs more slowly, and their learning criterion was set to a lower value on both tasks. Sleep-dependent gains in retention as expressing themselves at recall performance probably strongly depend on pre-existing knowledge about the task stimuli, i.e., schemas and concepts related to the meaning of the words acquired on the paired associate task and to the pictures of the 2D object location task (Schneider & Bjorklund, 1992; Bjorklund & Schneider, 1996; Tse et al., 2007). Less elaborate schemas, as present in children, may slow down sleep-dependent consolidation and assimilation of new memories to these schemas, thereby masking the benefits of increased SWS in this age group.

Sleep-dependent changes in skills were the target of two previous developmental studies in humans which, in fact, agree with the present findings of influences in opposite direction of post-training sleep in children and adults. Gomez et al. (Gomez et al., 2006) familiarized 15 – month old infants with an artificial language, and 4 hours later examined retrieval by measuring the time the infant oriented towards the same or novel strings of words. Compared to a non-napping control group, children who had napped after learning, at retrieval test appeared to be more able to abstract a rule-like pattern from the learned

structures. Of note however, signs of remembering the exact word dependencies were significantly enhanced in the wake group compared with the napping infants. In a sample of 9-11 years old children, sleep after training on a SRTT impaired implicit knowledge of the underlying sequence, as assessed by reaction time differences to cue positions that do or do not adhere to an underlying grammar (Fischer et al., 2007). Following the wake retention period implicit SRTT knowledge remained almost unchanged. This pattern clearly differed from that in adults who improved in implicit sequence knowledge across overnight sleep but showed deteriorated performance after a retention period of daytime wakefulness. This pattern bears distinct similarities with the present findings based on explicit motor sequence learning, where children did not show a gain of skill overnight, but did so across daytime wakefulness. In combination with equivalent findings in young birds learning a song (Deregnacourt et al., 2005), these findings strongly speak for the notion that sleep during development exerts a specific effect on the offline learning of a skill that differs from that in adults and manifests itself in a relatively impaired performance at retrieval, if compared with performance after a wake control interval.

Unspecific factors like tiredness, motivation and mood are unlikely to have confounded our results since assessment of these factors by self-ratings and standardized interviews did not indicate any difference between sleep and wake conditions in the children or adults. Due to portable EEG devices subjects could sleep in their regular home environment and they were also adjusted to the recordings by an adaptation night. Children and adults slept according to their habitual bed time and testing in the morning always took place not until ~60 minutes after awakening. These procedures safely exclude that unfamiliarity with the experimental conditions or sleep inertia during testing substantially or selectively influenced memory performance in the sleep condition. Importantly, if such factors exerted an influence in children, the consolidation of declarative memory should have been also affected.

Since it is not appropriate to experimentally deprive young children from nocturnal sleep, we compared effects of sleep and wakefulness during periods covering, respectively, daytime and night-time. Thus, circadian rhythm might have biased our results. Although this confound cannot be entirely excluded, it seems unlikely to be substantial in light of the fact that at learning, performance on all tasks was closely comparable when taking place in the evening and morning (on the sleep and wake conditions, respectively). Likewise previous studies did not provide any hint that time of day at learning or retesting affected performance

on declarative or procedural tasks similar to those of the present study (Robertson et al., 2004; Gais et al., 2006; Fischer et al., 2006).

An immediately impairing effect of sleep or a lack of overnight gains in skill does not necessarily exclude an improving influence of post-training sleep on skill memory on the long term. The deterioration of song structure observed in the morning after post-training sleep in young birds vanished when the birds again trained the song, with a gain of structure developing towards the end of the day (Deregnacourt et al., 2005). Notably, birds that showed stronger post-sleep deterioration during development achieved a better final imitation at the end of the 3-months study epoch. Those authors considered the sleep-associated oscillation in song performance, which was not observed in adult birds, a reflection of competing demands of plasticity and consolidation specific to developmental learning, whereby sleep transiently reduces coherence of memory traces allowing for continuously reshaping of song structure through the correction of inappropriately consolidated structure during wakefulness. It remains to be elucidated, whether an oscillating time course is likewise a characteristic feature of skill learning during human development.

The mechanism mediating the specific effects of sleep on procedural memory consolidation during development is obscure. Delayed maturation of brain structures underlying procedural memory is unlikely to explain this finding, because the most relevant brain structures (like basal ganglia and primary motor cortex) mature earlier than those underlying declarative memory, and appear to be almost fully developed at the age of 3 years (Gogtay et al., 2006; Sowell et al., 2002; Casey et al., 2005; Gogtay et al., 2006).

However, lacking sleep-dependent gains in motor skill during development might result from an interaction between procedural and hippocampus-dependent declarative memory systems. There is increasing evidence that formation of procedural memories is not achieved as independently from hippocampal function as originally assumed. Particularly, at initial stages of motor skill acquisition, explicit mechanisms involving hippocampal function may interfere with implicit procedural aspects of task performance thereby slowing response speed (Jimenez, Mendez, & Cleeremans, 1996; Willingham, 1998; Poldrack et al., 2001; Poldrack & Rodriguez, 2003). Neuroimaging studies have demonstrated that skill acquisition can be accompanied by competitive interference between respective striato-frontal and hippocampal systems (Poldrack et al., 2001; Poldrack et al., 2003; Schendan et al., 2003; Forkstam & Petersson, 2005; Foerde, Knowlton, & Poldrack, 2006). The competitive interaction most likely extends to post-learning consolidation processes. Brown and Robertson (2007) showed that consolidation of motor skill can be enhanced by post-learning

performance on a declarative task considered to suppress competing declarative aspects of skill acquisition by retroactive inhibition. In adults, sleep-associated consolidation of declarative memories has been found to interfere with consolidation of procedural memories as well (Wagner et al., 2004; Fischer et al., 2006). Subjects who had gained explicit knowledge of the sequence grammar in an SRTT at retesting after post-training sleep, did not show the expected sleep-dependent speeding of reaction times to grammatical cue positions.

In this context, it is plausible that the lack of any sleep-dependent overnight gain in motor skill observed in our children likewise reflects competitive interference from hippocampus-dependent declarative memories. There is evidence that learning a procedural motor task, like the SRTT, involves hippocampal recruitment in addition to striatal activation in children as well (Thomas et al., 2004). Aspects of task performance encoded in hippocampal networks may become particularly strengthened during subsequent sleep in children, because their sleep is rich of SWS known to preferentially support (hippocampus-dependent) declarative memory consolidation (Plihal et al., 1997; Peigneux et al., 2004; Marshall et al., 2006; Marshall et al., 2007). On the background of minor behavioral automation reached during learning the enhancement of hippocampus-dependent explicit aspects of a task during sleep may well act to prevent delayed gains in speed of motor skill.

After subjects were retested on the learned sequence, they performed on a novel sequence. In comparison with explicit learning of declarative tasks, learning of procedural skill is thought to be highly specific to the task parameters showing little generalization to similar tasks (Karni et al., 1998). Concurring with this conceptualization, adults showed a sleep-dependent gain of skill specifically for the trained sequence whereas performance on the novel sequence remained unchanged. In contrast, the relatively impaired performance on the learned sequence after sleep in children appeared to generalize to the novel sequence which after sleep was performed also more slowly than after the wake retention interval. (Note, general motor inertia after sleep cannot account for this effect because initial learning performance in the children was closely comparable in the morning after sleep and in the evening after daytime wakefulness.) Thus slowing of responses to both the learned and novel sequence at retrieval testing after sleep suggests that explicit mechanisms of behavioural control were installed that biased sequence tapping in a more general way.

This view does not contradict findings indicating that in younger children a learned finger tapping sequence is less susceptible to interference by training a different sequence 2 hours later (Dorfberger et al., 2007). On the contrary, those data showing more effective consolidation of motor representations during wakefulness immediately following training in

younger than older children, concur with our results of distinct performance gains in the children after the wake interval. In combination, these data indicate that during developmental learning major aspects of procedural memory consolidation can take place in the wake state. However, as to hippocampus-dependent declarative memory our data demonstrate a benefit from sleep in children similar to that in adults. We assume it is the contribution of hippocampus-dependent consolidation processes whereby sleep, compared to a wake retention condition, weakens new motor skill memories. The utmost importance of skill learning during development makes this view an intriguing issue of future research.

# **Study 2 – The role of sleep in the extraction of explicit knowledge from an implicitly learned motor task in children and adults**

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## **Introduction**

Adult humans have unique capabilities to regulate behavior based on an insightful understanding of complex situations, i.e. on explicit knowledge about their environment. Explicit knowledge does typically not arise immediately upon encountering a complex situation, but rather results from a gradual gain of insight. In this process, representations of the complex stimulus patterns initially encoded implicitly and without full awareness, become transformed and restructured such that invariant and relevant features of these representations are enhanced to eventually enter consciousness (Cleeremans, 2008). This process of restructuring representations in memory that can promote the extraction of explicit knowledge from implicitly encoded information is supported by sleep (Wagner et al., 2004; Yordanova et al., 2008; Fischer et al., 2006; Walker, 2009; Payne et al., 2009). In fact, the implicit-to-explicit conversion of knowledge has been proposed to be part of a system consolidation process occurring during slow wave sleep (SWS), in which newly encoded representations stored temporarily in hippocampal networks are reactivated to become redistributed to neocortical networks for long-term storage (Diekelmann et al., 2010). Adults outperform children in virtually all cognitive domains. However, sleep is deeper in children and they show distinctly greater amounts of SWS. Therefore, we aimed to test in children whether sleep can facilitate the gain of explicit knowledge about rules and regularities underlying complex stimulus situations. Furthermore, we searched for the neuronal correlates of explicit knowledge after retention sleep in a supplementary fMRI study.

## Methods

**Participants.** Forty-seven healthy children between 8–11 years (mean  $\pm$  SEM:  $9.56 \pm 0.15$  yrs) and twenty-nine adults ( $23.54 \pm 0.58$  yrs) participated in the main experiment. Twenty-six children ( $9.56 \pm 0.28$  yrs) and thirty-two adults ( $26.32 \pm 0.72$  yrs) participated in the supplementary fMRI study. The participants were recruited via advertisements placed at the university and local after-school care clubs. Interviews with the parents and children as well as standardized questionnaires ensured that the children had no behavioural problems, cognitive impairments or sleep disorders. Children as well as adults had no history of any neurological or psychiatric disorder and did not take any medication at the time of experiments. All participants were right handed. We carefully surveyed the children's and adults' sleep schedules in order to adapt bedtimes during the study to the participants' habitual bedtime. All participants of the main experiment were adapted to polysomnographic recordings on a night preceding the experiment proper. The study was approved by the local ethics committee and participants as well as the children's parents gave informed consent before participation. In the main experiment, data from three participants (two children, one adult) did not enter the sleep analyses due to artefacts in the EEG. Data from one child and one adult were excluded from the fMRI analyses in the supplementary experiment because of scanner artefacts.

**Design and Procedure.** In the main experiment, children and adults were randomly assigned to a 'Sleep' group and, a 'Wake' group, respectively. All participants performed on the motor sequence learning task ('button-box task' described below) at their home environment and they also slept at home. In the Sleep group, the learning phase of ~15 min took place between 7:00 – 9:15 PM in the children and between 10:00 -12:15 PM in the adults. Thereafter, participants of both age groups went to bed, so that lights were turned off at the habitual bedtime, which for the children was between 7:30 and 9:30 PM, and for the adults between 10:30 and 12:30 PM. In the next morning participants were awakened at their usual time. Retrieval testing took place ~60 minutes later. The interval between learning and retrieval testing averaged 11 hours. In the Wake group learning took place in the morning ~60 min after awakening from night-time sleep and retrieval was tested after a retention interval of wakefulness that also lasted ~11 hours. During the wake retention interval participants followed their daily schedules. The parents kept a continuous record of the children's activities in order to control for possibly disturbing or stressful events or interfering cognitive activities. Adult participants kept this record themselves. Before learning and retrieval testing

in both conditions participants estimated their subjective tiredness and motivation. Children did so by oral report and adults filled in a standardized questionnaire. An additional control group of children learned the task in the evening (i.e. like the Sleep group) but retrieval was tested immediately after the learning phase.

The additional fMRI study was similar except that learning and retrieval testing took place in a slightly modified version (see below) inside the MR scanner, and no polysomnographic recordings were performed when participants slept at home. Sleep duration and quality on the night after learning were assessed by verbal report and a questionnaire in children and adults, respectively.

**Memory Task.** To investigate memory consolidation we used the ‘button-box task’ which is an implementation of a motor sequence learning task specifically adapted to the motor abilities of children. The button box is a white 50 cm x 22 cm x 7 cm box with eight coloured buttons placed on its upper panel in two rows that are consecutively flashed up according to a repeating 8-elements sequence. Participants were instructed to press the button flashing up as fast as possible with the non-dominant (left) hand. Pressing the respective button turned off the illumination, and the next button flashed up immediately afterwards. At learning, participants performed 10 blocks (each including five eight-element sequences), and after each block a short break was made to provide the participant with a feedback (on a computer screen) about the mean reaction time during the preceding block. For implicit learning, the speed of button press responses was analyzed as mean reaction time in each block.

To assess (at retrieval testing) explicit knowledge of the sequence trained on the button-box task participants stood in front of the button-box and were asked to recall the sequence by pointing at the buttons in the same order as they flashed up during the learning phase. As a measure of explicit sequence knowledge the number of correct transitions, i.e., of the correct recall of two buttons in a row was used, yielding a possible maximum score of eight.

In the fMRI experiment, the button-box task was adjusted to the specific requirements of the MR environment, i.e., to reduce movement artefacts the button-box and the buttons were constructed smaller with the distance between the buttons being also shorter (panel size 24 cm x 12 cm). The task procedure at learning was the same as that used in the main experiment, except that the feedback about reaction times after each block was given orally by the experimenter. At retrieval testing, six blocks of sequence recall alternated with six blocks of a control task, with each block including eight trials. Sequence and control blocks were

separated by a 20-sec interval during which the participant was informed about the type of task (sequence recall versus control task) in the upcoming block. Each trial started with flashing one of the eight buttons of the button-box for 4 sec. For the blocks of sequence recall, the participants were asked to imagine the two buttons succeeding this button in the sequence they had trained in the learning phase. After 4 sec the button illumination turned off, and only then participants were allowed to press the two next buttons of the sequence. The next trial started with a variable inter-trial interval of 4-10 sec. The amount of explicit knowledge was indicated by the number of correctly retrieved sequence triplets. In the control blocks, participants were asked to press two of the buttons adjacent to the flashed button as soon as the button light turned off after 4 sec. Because of the task modifications, behavioural performance during sequence recall blocks could not be directly compared with retrieval performance in the main experiment. Hence, explicit sequence knowledge was additionally tested in these participants after scanning using the same free recall procedure as in the main experiment.

**Analysis of Behavioural Data.** Statistical analysis of reaction times during implicit learning was based on 2x2x2 analyses of variance (ANOVA) including the two group factors ‘Age’ (children versus adults) and ‘Sleep/Wake’ representing the two kinds of retention intervals, and a repeated measures factor representing the first and last block of training. Retrieval of explicit sequence knowledge after the retention interval was analyzed using a 2 (Age) x 2 (Sleep/Wake) ANOVA. Post-hoc comparisons as well as comparisons of sleep parameters between children and adults were performed using t-tests. Sleep parameters were correlated with explicit sequence knowledge after retention sleep using Pearson’s correlation coefficients.

**Sleep and EEG Recordings.** Standard polysomnographic recordings were obtained in the main experiment using a portable amplifier (SOMNOscreen EEG 10-20, Somnomedics, Kist, Germany). EEG signals were sampled at a frequency of 256 Hz and filtered between 0.03 and 35 Hz. Recordings were visually scored offline according to the criteria by Rechtschaffen & Kales (Rechtschaffen et al., 1968). For each night, sleep onset, total sleep time, and the time as well as the percentage of total sleep time spent in the different sleep stages were determined. Sleep stages are wake, Non-Rapid Eye Movement (NonREM) sleep stages 1, 2, 3, and 4, slow wave sleep (SWS, i.e., the sum of stage 3 and 4 sleep) and REM sleep. Sleep onset latency (i.e., the first occurrence of a period of stage 1 sleep followed by stage 2 sleep)

was determined with reference to the time of lights off. Latencies of SWS and REM sleep were determined with reference to sleep onset.

Power spectral analysis of the EEG signal was performed using Fast Fourier Transformation on all recording sites (F3, F4, C3, C4, P3, P4) and separately for periods of NonREM sleep (sleep stages 2, 3 and 4) and REM sleep. The spectra were calculated for successive 8-sec (2048 data points) artefact-free intervals using a Hanning window to taper the data. Power density ( $\mu\text{V}^2/\text{Hz}$ ) was computed for three standard frequency bands of interest, i.e., for slow wave activity (SWA, 0.6–4 Hz), theta activity (5–8 Hz), and spindle activity (11–15 Hz). For statistical analyses, power values were log transformed and subjected to ANOVA that basically included factors representing the three Frequency bands and the two Age groups.

In the Sleep groups of the additional fMRI experiment, adult participants themselves kept a record about sleep duration and quality in the night after the learning phase. For children, their parents kept this record. On these nights, children slept on average  $9.6 \pm 0.3$  hours and woke up  $0.7 \pm 0.3$  times per night. Adult participants slept  $8.0 \pm 0.76$  hours and woke up  $1.2 \pm 0.3$  times per night.

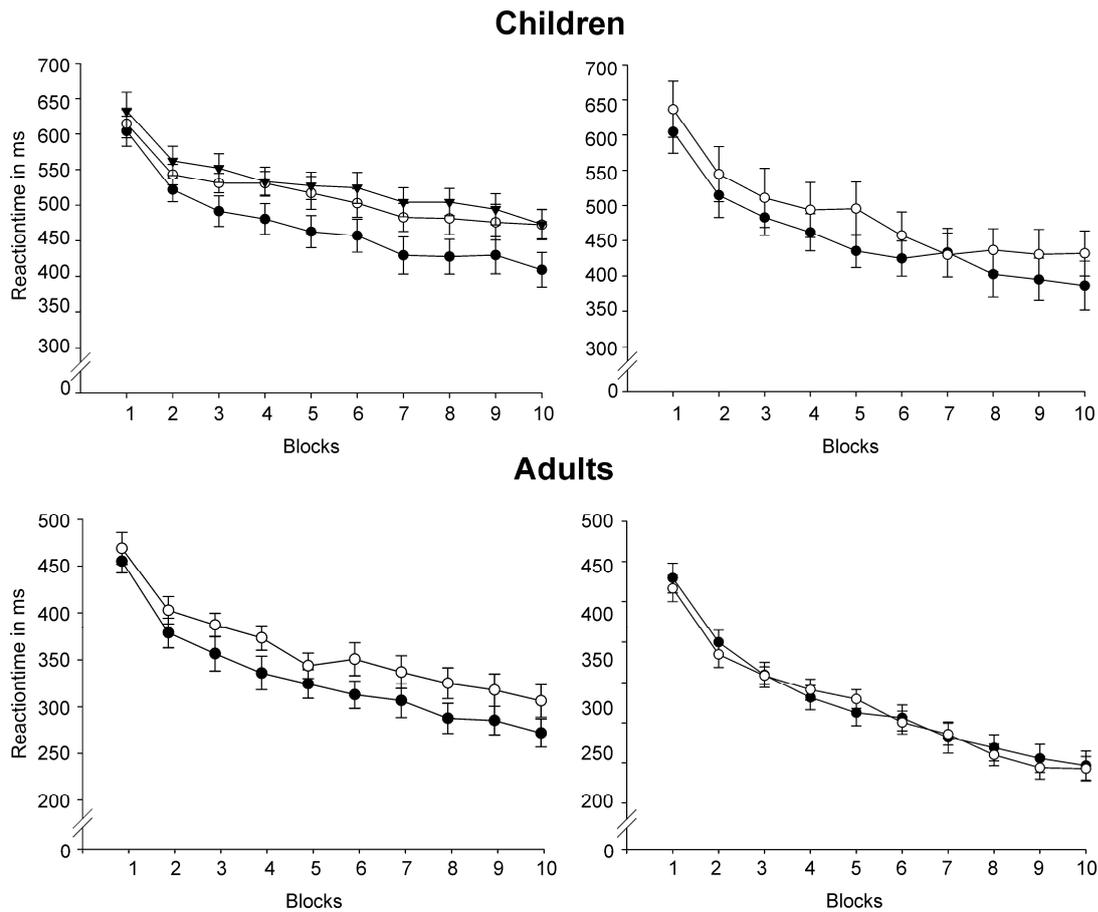
**fMRI Data Acquisition and Processing.** Functional imaging was performed on a 3 T Siemens Trio MR scanner with a 12 channel phased array head coil. 40 axial slices were acquired using an echo-planar (EPI) T2\* weighted imaging sequence with a voxel size of  $2 \times 2 \times 3$  mm (TR = 2.56 s, TE = 30 ms, flip angle  $90^\circ$ , FoV 208 mm<sup>2</sup>, matrix  $104 \times 104$ ).

The five initial scans were discarded from the analysis to account for magnetic saturation effects. Preprocessing and data analysis was performed using Statistical Parametric Mapping (SPM8, Wellcome Department of Cognitive Neurology, London, UK) under Matlab R2008a. Images were realigned, normalized into standard anatomical space (MNI), and smoothed with a Gaussian kernel of 6 mm full width at half maximum (FWHM). For each participant, evoked hemodynamic responses to event types were modelled with a delta (stick) function corresponding to stimulus presentation convolved with a canonical hemodynamic response function within the context of a general linear model. Additionally the data were filtered using a 128-sec cut-off high pass filter to account for low-frequency drifts. Linear contrasts were used to analyze main effects and interactions of the two factors Sleep/Wake and Age. Areas of interest for small volume correction (10 mm sphere) were taken from two previous studies focusing on effects of sleep and wakefulness on consolidation of motor sequences (Albouy et al., 2008; Debas et al., 2010) including the following coordinates:

striatum [23 4 -5] [-15 15 0], insula [30 11 -15] and hippocampus [40 -26 -20]. Additionally, for an exploratory analysis of the whole brain a threshold of  $P < 0.05$  (FWE-corr - Family-wise error rate-corrected) in a minimum number of 3 adjacent voxels was used for the contrast Recall Trials > Control Trials.

## Results

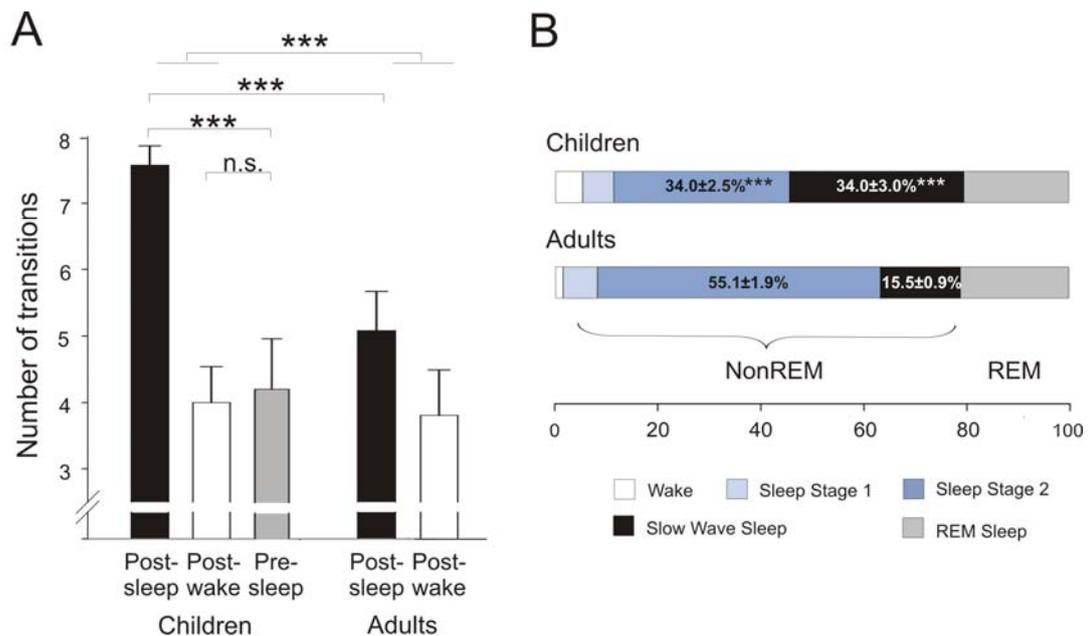
**Implicit Learning Performance in both experiments.** In the main experiment, performance on the button-box task significantly improved across the training blocks in both children and adults ( $F(3.5,241.2) = 137.76$ ;  $P < 0.001$ , for main effect of Block; Figure 7). As expected, reaction times in children were generally slower than in adults ( $F(1,69) = 77.8$ ;  $P < 0.001$ ). However, the improvement in performance from the first to the last block of training was comparable for both age groups ( $F(3.5,241.2) = 0.68$ ;  $P = 0.59$ , for Age by Block interaction). Importantly, training performance did not differ between the Sleep and Wake groups neither in children nor in adults (children:  $F(2,42) = 2.36$ ;  $P = 0.11$ ; adults:  $F(1,27) = 2.20$ ;  $P = 0.15$ , for main effect of Sleep/Wake). Learning performance in the supplementary fMRI experiment revealed basically the same results ( $F(3.69,195.6) = 142.79$ ,  $F(1,53) = 46.82$ ;  $P < 0.001$ , for main effects of Block and Age;  $P > 0.36$ , for all other main effects and interactions; Figure 7). Error rates were generally very low ( $< 1.3$  per block) and did not differ systematically between age groups, blocks or sleep vs. wake retention intervals ( $P > 0.32$ , for all relevant effects).



**Figure 7.** Mean ( $\pm$  s.e.m.) reaction times (in milliseconds) on the ten blocks of the button box task during the learning phase in children (upper panels) and adults (lower panels) in the main experiment (left panels) and the additional fMRI study (right panels). Sleep groups - black circles; Wake groups - empty circles; Control group of children tested for explicit sequence knowledge directly after training blocks - black triangles.

**Explicit Sequence Knowledge in the Main Experiment.** In comparison with the wake retention interval group, sleep produced a significant increase in explicit knowledge of the motor sequence independent of age ( $F(1,58) = 18.33$ ;  $P < 0.001$ , Sleep/Wake main effect). The benefit from sleep was most remarkable in children who almost all perfectly recalled the eight-element sequence after sleep (Sleep:  $7.57 \pm 0.29$  correctly remembered transitions, Wake:  $4.00 \pm 0.54$ ,  $t(31) = 5.30$ ;  $P < 0.001$ ; Figure 8A) whereas in the adults the sleep-associated increment in explicit sequence knowledge was distinctly less pronounced and not significant (Sleep  $5.08 \pm 0.59$  transitions, Wake  $3.81 \pm 0.68$ ,  $t(27) = 1.36$ ;  $P = 0.19$ ;  $F(1,58) = 4.71$ ;  $P = 0.046$ , for Sleep/Wake by Age interaction). Indeed, explicit knowledge after sleep was distinctly better in children than in adults ( $t(25) = 3.86$ ;  $P = 0.001$ ). In contrast, after the wake interval, free recall of the motor sequence, although on average slightly better in

children, did not significantly differ between age groups ( $t(33) = 0.22$ ;  $P = 0.83$ ). Also, the gain of explicit sequence knowledge after sleep in children was highly significant in comparison with performance of a control group of children asked to freely recall the motor sequence directly after the evening training ( $4.36 \pm 0.71$  correctly remembered transitions,  $t(26) = 4.2$ ;  $P < 0.001$ ), whereas performance after the wake retention interval did not differ from this control group ( $t(31) = 0.41$ ;  $P = 0.68$ ).



**Figure 8.** (A) Explicit sequence knowledge as indicated by the number of correctly recalled element transitions was enhanced after retention periods of sleep (black bars) in comparison with wakefulness (white bars) and this benefit from sleep was strikingly greater in children than in adults (see text for ANOVA results). The grey bar indicates explicit sequence knowledge in a control group of children tested immediately after learning. (B) Percent time spent in the different sleep stages (wake - white, stage 1 sleep – light blue, stage 2 sleep – dark blue, slow wave sleep (SWS) - black, rapid eye movement (REM) sleep - grey) for children (upper bar) and adults (lower bar).

**Explicit Sequence Knowledge in the fMRI Study.** At recall in the scanner participants were asked to respond, with a delay of 4 sec, to an illuminated cue button by pressing the two buttons that had succeeded the cue button in the originally trained sequence. In the control task they should press, again with a delay of 4 sec, two buttons adjacent to the cue button. The number of correct sequence triplets was calculated for both recall trials and control trials. The analysis confirmed that in control trials, participants did not press any buttons related to the trained sequence (i.e., the mean number of sequence-associated triplets was  $<1$ ). On recall

trials, participants pressed on average a greater number of correct sequence triplets after retention periods of sleep than of wakefulness (children: Sleep  $21.92 \pm 5.77$ , Wake  $13.69 \pm 5.11$ ; adults: Sleep  $25.13 \pm 5.18$ , Wake  $18.65 \pm 4.46$ ) although this difference did not reach significance ( $F(1,54) = 2.06$ ;  $P = 0.16$ , for main effect of Sleep/Wake). However, due to the task modifications that were introduced to enable fMRI imaging, motor performance during recall blocks in the scanner did not as sensitively and validly assess explicit sequence knowledge as the free recall procedure used in the main experiment. For this reason, we tested retrieval of explicit sequence knowledge again after scanning using exactly the same procedure as in the main experiment. Results from this test confirmed that explicit knowledge about the sequence structure was greater when participants had slept after implicitly learning the motor sequence than when they had stayed awake ( $F(1,54) = 3.41$ ;  $P = 0.035$ ). Post-hoc contrasts revealed that the difference between Sleep and Wake groups was significant in the children (Sleep:  $5.53 \pm 0.70$ , Wake:  $3.38 \pm 0.84$ ,  $t(24) = 1.97$ ;  $P = 0.03$ ) but not in the adults (Sleep:  $4.93 \pm 0.76$ , Wake:  $4.29 \pm 0.71$ ,  $t(30) = 0.62$ ;  $P = 0.27$ ;  $F(1,54) = 1.00$ ;  $P = 0.16$ , for interaction Sleep/ Wake x Age; all tests one-tailed).

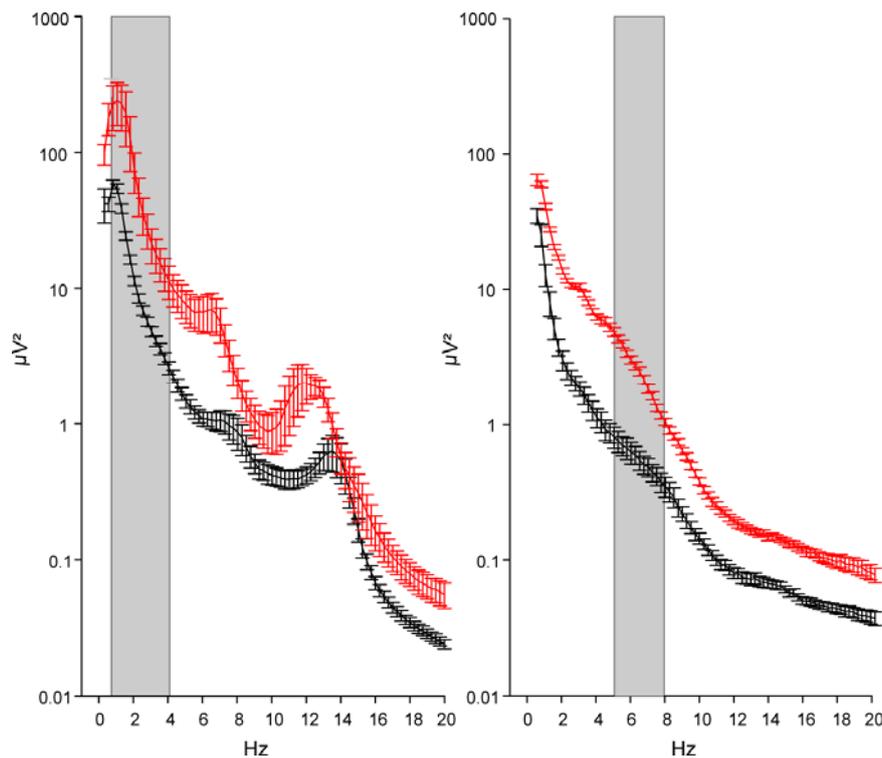
**Sleep Data in the Main Experiment.** Sleep was recorded during the night after learning in the Sleep groups (see table 2 for a summary of polysomnographic results). Children spent three times more time in SWS than adults ( $203.2 \pm 19.3$  vs.  $61.2 \pm 4.9$  min,  $t(22) = 7.13$ ;  $P < 0.001$ ; Fig. 8B). The percentage of total sleep time the children spent in SWS was also more than twice as high as in the adults ( $t(22) = 5.94$ ;  $P < 0.001$ ), although children slept on average 204.9 min longer than adults ( $t(22) = 9.1$ ;  $P < 0.001$ ). The increase in SWS was associated with a shorter latency of SWS in children than in adults ( $t(22) = -5.96$ ;  $P < 0.001$ ). On the other hand, the percentage of REM sleep was closely comparable in both groups ( $20.5 \pm 1.0$  vs.  $20.6 \pm 1.3$  %,  $t(22) = -0.04$ ;  $P = 0.96$ ).

**Table 2. Sleep Data**

	Children	Adults
Total Sleep Time (min)	598.5 ± 15.0	393.6 ± 16.8
Sleep onset (min)	8.5 ± 1.0	12.8 ± 3.7
SWS latency (min)	9.21 ± 0.9	20.9 ± 1.7
REM latency (min)	129.3 ± 13.1	99.0 ± 11.3
<b>Sleep stages - time in min</b>		
Wake	31.2 ± 3.4	5.3 ± 1.4
Stage 1	36.6 ± 4.2	25.3 ± 2.7
Stage 2	202.0 ± 15.6	218.0 ± 8.0
SWS	203.2 ± 19.3	61.2 ± 4.9
REM	124.0 ± 8.5	82.8 ± 7.9
<b>Sleep stages - % of TST</b>		
Wake	5.3 ± 0.6	1.3 ± 0.3
Stage 1	6.1 ± 0.7	6.3 ± 0.6
Stage 2	34.0 ± 2.5	56.1 ± 2.2
SWS	34.0 ± 3.0	15.5 ± 0.9
REM	20.5 ± 1.0	20.6 ± 1.3

Mean ( $\pm$  s.e.m.) total sleep time (TST), latency of SWS and REM sleep (in minutes, with reference to sleep onset) and time spent awake, in stage 1 sleep, stage 2 sleep, slow wave sleep (SWS) and rapid eye movement (REM) sleep in minutes and percentage of total sleep time. Left column indicates p-level for statistical comparisons by t-test between the age groups.

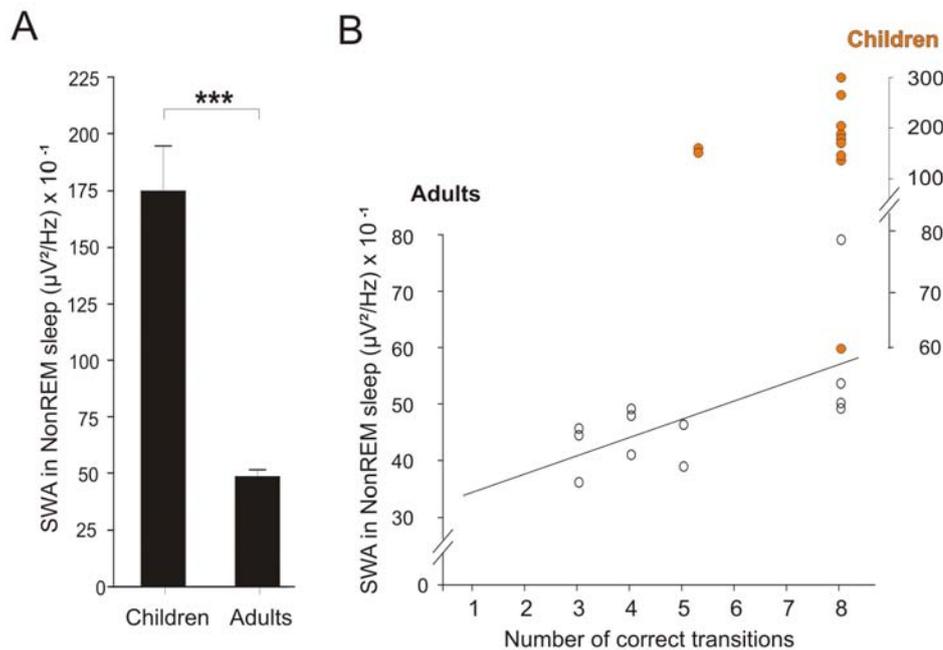
Power spectral analyses of the EEG recorded during NonREM sleep from frontal electrode sites revealed that power density was significantly higher in children than in adults in all three frequency bands of interest with an emphasis of the 0.6-4 Hz slow wave activity (SWA) frequency band ( $t(21) = 6.07$ ;  $P < 0.001$ ; Figure 10A), whereas increases in power in the theta (5-8 Hz,  $t(21) = 4.18$ ;  $P < 0.001$ ) and spindle frequency bands (11-15 Hz,  $t(21) = 2.98$ ;  $P = 0.007$ ), although quite consistent, seemed somewhat smaller ( $F(1.0,21.1) = 37.35$ ;  $P < 0.001$ , for Frequency band x Age interaction; Figure 9). The differences between the NonREM sleep EEG in children and adults were likewise obtained for recordings from central and parietal positions.



**Figure 9.** Mean ( $\pm$  s.e.m.) EEG power ( $\mu\text{V}^2$ , log scaled) during NonREM sleep (stages 2 and slow wave sleep, left panel) and REM sleep (right panel) in children (red lines) and adults (black lines). Data from frontal recordings (F4) are illustrated and represent averages across all artefact-free intervals of an entire night. Shaded grey areas indicate the frequency bands (slow wave activity - 0.6-4 Hz and theta activity - 5-8 Hz) that were revealed to be significantly associated with explicit sequence knowledge ( $r = 0.64$ ,  $r = 0.61$ , respectively, see main text).

Because our children showed nearly perfect motor sequence knowledge after sleep, i.e., a ceiling effect (Figure 10B), correlations of EEG activity with recall performance after sleep could not be calculated for this group. However, in adults, SWA during NonREM sleep was significantly associated with explicit knowledge of the motor sequence after sleep, in particular from right frontal recordings ( $r = 0.64$ ,  $P = 0.026$ ; respective correlations for central and parietal sites were  $r = 0.55$  and  $0.14$ ;  $P = 0.063$ ,  $P = 0.66$ ; Figure 10B). There were no other correlation between EEG activity and memory performance in adults, except that frontal theta power during REM sleep was also positively correlated with retrieval of explicit sequence knowledge ( $r = 0.61$ ;  $P = 0.035$ ). As frontal theta activity during REM sleep was highly correlated to frontal SWA during NonREM sleep ( $r = 0.75$ ;  $P = 0.005$ , for F4), and REM sleep follows NonREM sleep in the normal sleep cycle, this pattern points to a sequential function of these sleep stages in memory processing with REM sleep possibly

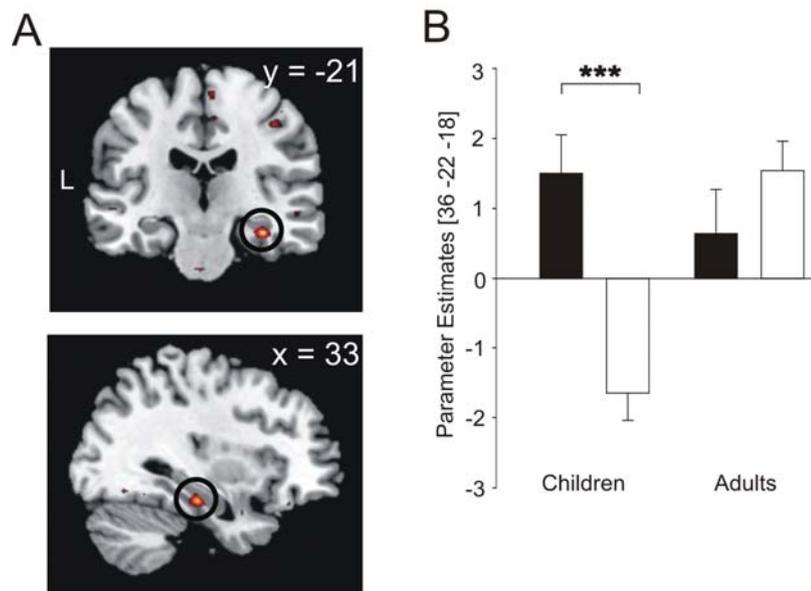
adding to the stability of memory representations that were reorganized during SWS (Diekelmann et al., 2010b; Maquet, 2001).



**Figure 10.** (A) SWA during post-training NonREM sleep was distinctly greater in children than in adults ( $P < 0.001$ ). (B) In adults (empty circles), SWA during NonREM sleep was positively correlated with explicit knowledge during sequence recall after sleep. Because of the ceiling effect in children (orange circles), i.e., almost all showed perfect explicit sequence knowledge after sleep, a correlation with SWA could not be determined for this group. Note, broken y-axis in children; recordings from F4.

**Brain activation during explicit sequence retrieval.** As expected from previous studies (Penhune et al., 2002; Rauch et al., 1997), retrieval of sequence knowledge compared with control trials induced activation in several prefrontal cortex areas and closely connected striatal areas (recall trials > control trials; all  $P_{\text{FWE}} < 0.05$ , Table 3). For the Sleep > Wake contrasts significance is corrected for small volumes of interest (SVC) centered on peaks taken from previous imaging studies (Albouy et al., 2008; Debas et al., 2010) which examined the consolidation of motor sequences during sleep: <sup>a</sup>striatum [23 4 -5], <sup>b</sup>striatum [-15 15 0], <sup>c</sup>insula [30 11 -15], <sup>d</sup>hippocampus [40 -26 -20]. Independent of the age factor, retrieval of explicit sequence knowledge after sleep induced higher BOLD responses than after the wake period in brain regions known to be associated with motor learning (Doyon et al., 2005), i.e., the basal ganglia and the cerebellum (Sleep > Wake; all  $P_{\text{SVC}} < 0.05$ , Table 3). Compared with adults, in children explicit retrieval of sequence knowledge after sleep was associated with a

higher BOLD response (recall trials > control trials) in the right anterior hippocampus [36, -22, -18] (Sleep > Wake x Children > Adults,  $P_{SVC} = 0.023$ ; Table 3; Fig. 11A,B).



**Figure 11.** (A) During retrieval of explicit sequence knowledge after retention sleep the right anterior hippocampus was activated to a greater extent in children than in adults (thresholded at  $P_{SVC} < 0.05$ ). (B) Respective parameter estimates at the coordinates of local maxima. Means  $\pm$  s.e.m. are indicated; \*\*\* -  $P < 0.001$ , \*\* -  $P < 0.01$ . Note, values do not indicate absolute activation but relative increases during retrieval of learned sequences with reference to control task performance.

**Table 3.** Brain Activity during Explicit Sequence Recall

	MNI coordinates (mm)			Z score	P (FWE-corr)
	x	Y	z		
<b>Recall Trials &gt; Control Trials</b>					
L. Lingual Gyrus (BA 18)	-12	-82	-10	7.35	< 0.0001
L. Putamen, Lentiform Nucleus	-22	14	-4	7.32	< 0.0001
R. Nucleus Caudatus, Caudate Head	16	14	2	7.29	< 0.0001
L. Middle Frontal Gyrus (BA 9)	-42	12	30	5.72	0.0005
L. Precuneus (BA 19)	-38	-74	36	5.65	0.0007
R. Inferior Frontal Gyrus (BA 9)	40	6	28	5.47	0.0019
L. Middle Frontal Gyrus (BA 46)	-44	24	24	5.26	0.005
L. Middle Frontal Gyrus (BA 6)	-40	6	52	5.07	0.012
R. Medial Frontal Gyrus (BA 6)	4	-28	64	5.05	0.013
R. Paracentral Lobule (BA 6)	10	-32	58	4.96	0.02
R. Middle Temporal Gyrus (BA 39)	44	-64	20	4.98	0.018
L. Middle Temporal Gyrus (BA 39)	-42	-70	22	4.79	0.04
<b>Sleep &gt; Wake</b>					<b>P<sub>svc</sub></b>
R. Medial Globus Pallidus <sup>a</sup>	22	-6	-4	3.69	0.013
R. Ventral Putamen <sup>a</sup>	26	8	2	3.52	0.022
R. Ventral Putamen <sup>a</sup>	28	6	-8	3.43	0.028
L. Ventral Putamen <sup>b</sup>	-24	12	-4	3.44	0.027
R. Insula (BA 13) <sup>c</sup>	36	14	-10	3.37	0.033
<b>Children &gt; Adults x Sleep &gt; Wake</b>					
R. Anterior Hippocampus <sup>d</sup>	36	-22	-18	3.49	0.023

Brain regions showing significant activity during explicit sequence recall. For the contrast Recall Trials > Control Trials results of an exploratory whole brain analysis are indicated, thresholded at  $P < 0.05$ , FWE-corr - Family-wise error rate-corrected. Minimal voxel size  $k = 3$ .

## Discussion

Our finding that sleep enhances the extraction of explicit knowledge about a sequence structure which was implicitly learned before sleep, confirms several previous studies (Wagner et al., 2004; Yordanova et al., 2008; Fischer et al., 2006), and corroborates the notion of an active system consolidation process during sleep in which newly encoded memory representations undergo qualitative changes and restructuring that eventually promotes the conscious recollection of invariant structural features of these memories (Diekelmann et al., 2010). Most strikingly, sleep in children was distinctly more effective in producing explicit sequence knowledge than sleep in adults, which appears to be particularly

linked to the distinctly greater amounts of SWS in children. Conversion of implicit into explicit knowledge is associated with increased time spent in SWS in adults (Yordanova et al., 2008). Here, we found additionally in adults a significant correlation of retrieved explicit sequence knowledge with SWA dominating the EEG during SWS. Due to near optimal retrieval performance such correlations could not be calculated in our children who showed also distinctly enhanced SWA. In fact, children around the age of 11 years show a developmental peak in SWA together with peaks in cortical grey matter density and synaptic density (Campbell et al., 2009; Buchmann et al., 2010). Maturational peak levels of SWA thus possibly reflect high synaptic density and synaptic efficacy as both factors boost neuronal synchronization (Esser, Hill, & Tononi, 2007) and might represent general prerequisites for flexible restructuring of memory representations during sleep.

Formation of explicit knowledge in the declarative memory system has proven to critically rely on structures in the medial temporal lobe and the prefrontal cortex in adults and also in children (Eichenbaum, 1999; Ofen et al., 2007; Ghetti et al., 2010). Concurring with these findings, our data show that greater explicit sequence knowledge in children is associated with greater activation in the anterior hippocampus. The hippocampus is centrally involved in encoding of sequence structure, independent of whether learned explicitly or implicitly, in adults (Schendan et al., 2003; Albouy et al., 2008; Henke, 2010) and children, whereby initially children appear to recruit less hippocampal activity during implicit sequence learning than adults (Thomas et al., 2004). Furthermore, there is ample evidence indicating that slow oscillations drive repeated reactivations of newly encoded hippocampal memories (Ji et al., 2007; Marshall et al., 2006) thereby preferentially promoting formation of explicit memories within the cortico-hippocampal system (Diekelmann et al., 2010; Robertson et al., 2004). In previous fMRI studies, the hippocampus was revealed to be reactivated during SWS following learning of declarative memories and this region also showed greater activation in conjunction with the enhanced recall of declarative memories after sleep (Rasch et al., 2007; Gais et al., 2007). Hence, as sleep SWA was distinctly enhanced in children, it is tempting to speculate that the higher hippocampal activity during sequence recall in this group is a consequence of stronger SWA driven reactivations of hippocampal memories during sleep. Notably, capabilities for declarative memory formation appear to improve during childhood very much in parallel with SWA (Campbell et al., 2009; Buchmann et al., 2010; Ofen et al., 2007; Ghetti et al., 2010; Cycowicz, Friedman, Snodgrass, & Duff, 2001). Finally, the preferential SWA-driven formation of explicit memory could explain that children, unlike adults, do not show a sleep-dependent overnight gain in implicit motor sequence performance

competitively interacting with explicit memory formation (Wilhelm et al., 2008; Fischer et al., 2007; Poldrack et al., 2001).

We show here a striking capacity of sleep in children to promote the extraction of explicit knowledge from implicitly encoded information which is of fundamental adaptive importance. Humans acquiring a motor task are able to flexibly transfer their knowledge to other situations not until explicit knowledge representations are formed (Dienes et al., 1999; Seger, 1994). In general, the explicit recognition of invariant patterns and structures in the complexity of available information is a process that essentially determines the success of adaptation to changing environmental demands. It is this development of understanding in which children, because of their deeper sleep, can outperform adults.

# **Study 3 – Sleep-dependent consolidation of motor memories at different pre-sleep performance level in children and adults**

Submitted as: Wilhelm I, Metzkw M, Knapp S, Born J Sleep-dependent motor memory consolidation in children and adults: The pre-sleep level of performance matters.

## **Introduction**

Numerous studies have convincingly demonstrated in adults that sleep after practicing a new motor skill supports the consolidation of these skill memories (Walker et al., 2003a; Fischer et al., 2002; Wilhelm et al., 2008; Doyon et al., 2009). On a behavioral level, this consolidation manifests itself in greater gains in speed of motor performance across retention intervals containing sleep compared with wakefulness, despite the absence of any training during the retention interval (Walker et al., 2003a; Fischer et al., 2002; Debas et al., 2010). During early development, children acquire a great variety of basic procedural skills, and this period coincides with intense and long periods of sleep (Ohayon et al., 2004; Campbell et al., 2009). This makes it tempting to assume that the obviously high capabilities of learning in children are functionally related to their intense and superior quality of sleep. All the more astonishing is recent evidence that children unlike adults do not show sleep-dependent gains in procedural motor skills (Wilhelm et al., 2008; Fischer et al., 2007; Prehn-Kristensen et al., 2009) whereas processes of memory consolidation appeared to be even accelerated in time during wakefulness (Dorfberger et al., 2007). These findings agree with studies in young birds (zebra finches) learning a song, which likewise failed to exhibit any improvement in singing the tutored song after overnight sleep (Deregnaucourt et al., 2005). The mechanisms that could explain the lacking benefit for motor skill memories from sleep in children are presently entirely obscure.

Studies in adults have indicated that the pre-sleep performance level is one factor determining whether newly encoded procedural memories benefit from sleep or not (Diekelmann et al., 2009). In an oculomotor sequence task, sleep induced significant performance gains after a 24-hours retention interval only in fast learning subjects but not in slow learners (Albouy et al., 2008). On the other hand, in a finger sequence tapping task

sleep-dependent gains in reaction times occurred only for the difficult and unusual sequence transitions where subjects showed slow reaction times during learning (Kuriyama et al., 2004). In combination these results appear contradictory, inasmuch one of the studies showed sleep benefits only for fast responding subjects and the other only for slow responses during learning. However, general familiarity with the task procedures may be a modulating factor, as subjects are less familiar in learning an oculomotor sequence than tapping a finger sequence. Thus, it has been suggested that sleep benefits do not occur consistently in the beginning of learning a completely new skill and also when a skill is already highly over-trained, whereas robust sleep-dependent gains occur with intermediate levels of task performance (Stickgold, 2009).

Here, we addressed the question whether the lacking benefit from sleep for motor sequence memories that has been consistently observed in children in previous studies might result from their overall lower familiarity with and performance speed on these tasks. For this purposes we investigated the effects of different performance levels induced by varying the amount of pre-sleep training, on sleep-dependent motor memory consolidation in children (4-6 years) and adults using a coarse motor sequence task (i.e., the button-box task). Following previous suggestions (Stickgold, 2009) we hypothesized that, independent of age, sleep would preferentially affect motor sequence memories once an intermediate performance level is reached by the subject. Due to the great differences in performance levels between adults and children, to reach intermediate levels in children, they were extensively trained on the motor sequence task compared to controls who received a standard amount of training. By contrast, to induce this intermediate level of performance in adults, training was restricted to a minimum, compared with the standard training of controls.

## **Methods**

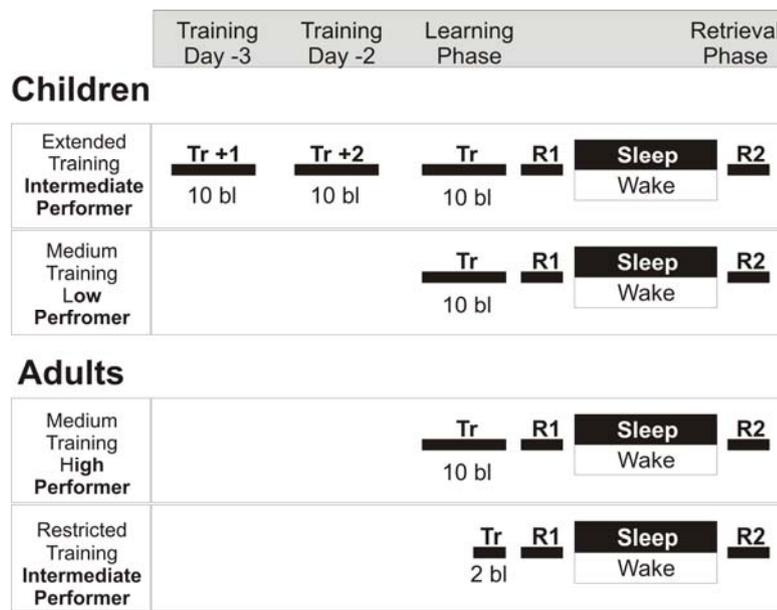
**Participants.** Thirty-five healthy children between 4–6 years (mean  $\pm$  SEM:  $5.44 \pm 0.75$  years) and 33 adults ( $24.80 \pm 3.96$  years) participated in the study. The participants were recruited via advertisements placed at the university and local kindergardens. Interviews with the parents and children as well as standardized questionnaires ensured that the children had no behavioural problems, cognitive impairments or sleep disorders. Children as well as adults had no history of any neurological or psychiatric disorder and did not take any medication at the time of the experiment. All subjects were adapted to polysomnographic recordings during

a nap preceding the experiments proper. The study was approved by the local ethics committee, and subjects gave written informed consent before participating. For the children this was accomplished by a parent. Additionally, all children provided verbal assent.

**Design and Procedure.** Subjects in each age group were randomly assigned to one of two experimental groups differing in the amount of training performed before the retention periods (i.e., low and medium amounts of training in adults, and medium and high amounts of training in children; Figure 12). For children and adults with medium amounts of training, this training (Tr) was part of the learning phase immediately before the retention interval, and consisted of ten blocks of performance on the button-box task, with each block including five 8-elements sequences. For children with high amounts of training, the training was extended by two times ten blocks (Tr +1, Tr +2) which were performed on two days preceding the day of the learning phase (in addition to the 10 blocks performed during the learning phase). The two days of additional training were separated from the learning phase by one day without any intervention. For adults with low amounts of training, the training during the learning phase was restricted to two blocks on the button-box task. By manipulating the amount of training, distinct performance levels could be induced before subjects entered the retention intervals of sleep and wakefulness: Children with medium amounts of training performed at lowest level before the retention interval ('low performers') whereas adults with the same amount of training performed best ('high performers'). Adults with low amounts of training as well as children with high amounts training both showed intermediate levels of performance at the end of the learning phase ('intermediate performers', Figure 13A).

Task performance at the end of a 10-blocks training is decelerated due to fatigue developing in the course of training. Thus, performance on the last blocks of training underestimates the actual performance level (Keisler et al., 2007). In order to achieve a more accurate baseline for evaluating sleep-dependent performance gains, our subjects performed on three further blocks of the button-box task 30 minutes after the training phase of the experimental session (Retrieval 1). The 30-minute delay period was used to fixate electrodes for polysomnographic recordings. Immediately after the first retrieval, the retention interval started which took ~120 min and in which subjects of the sleep groups took a nap. Subjects of the wake groups stayed awake during the entire retention interval. Naps took a maximum of 90 min, and subjects were awakened latest 30 min before the final retrieval test (Retrieval 2) in order to avoid any effects of sleep inertia on retrieval performance. In the wake conditions, the experimenter read books to the children or played games with them during the retention

interval, while adult subjects engaged in non-arousing and non-exhausting activities like watching TV or playing card games. The final retrieval test included three blocks of the button-box sequence. Before both Retrieval 1 and 2, subjects rated their actual mood, tiredness and motivation on 5-point (adults) and 3-point rating scales (children), respectively.



**Figure 12.** Experimental design.

**Memory Task.** To investigate memory consolidation we used the ‘button-box task’ which is a novel coarse motor sequence learning task specifically adapted to the motor abilities in children. The button box is a white 50 cm x 22 cm x 7 cm box with eight coloured buttons placed on its upper panel in two rows that are consecutively flashed up according to a repeating 8-elements sequence. Subjects were instructed to press the button flashing up as fast as possible. Pressing the respective button turned off the illumination, and the next button flashed up immediately afterwards. Each block during training and retrieval testing consists of five sequences with a 20-sec break between blocks during which the subject received feedback on his/her individual performance level during the preceding block. This was done by informing the children verbally whether their performance was better or worse than on the block before. In adults mean reaction time was displayed on the screen. The individual mean reaction times per block were also used for statistical analyses of motor sequence performance.

**Analysis of Behavioural Data.** Separate analyses of variance were run to evaluate (i) the level of motor sequence performance reached at the end of training, (ii) the improvement in motor performance from the end of training to the first retrieval (Retrieval 1) taking place immediately before the retention interval, and (iii) the gain in motor performance across the retention interval, i.e., from Retrieval 1 to Retrieval 2. The performance level reached at the end of training was indicated by the average reaction time across the last three blocks of the training period; in case of the adults with low amounts of training reaction times were averaged across the total 2 blocks of training they received. Statistical analyses of the performance levels relied on analyses of variances (ANOVA) basically including group factors for 'age' (children vs. adults), a repeated measures factor 'sleep/wake', and a group factor for the relative amount of 'training' each of these groups received, whereby in the children this amount was either 'intermediate' or 'high' and in adults it was either 'low' or 'intermediate', respectively. Additional analyses were run to evaluate the improvement in motor sequence performance across the training blocks with these ANOVA including a separate 'block' factor.

To analyze the improvement in motor performance across the two retention intervals (i.e., from the end of training to Retrieval 1 as well as from Retrieval 1 to Retrieval 2) percent differences were calculated, i.e., the difference between the individual average reaction times during the last three blocks of the training and at Retrieval 1, with performance at the end of training set to 100 %, as well as the difference between the individual average reaction times at Retrieval 1 and at Retrieval 2, with performance at Retrieval 1 set to 100 %. (For the adults with low amounts of training average reaction times were calculated for only two blocks.) The respective ANOVA included factors for age, sleep/wake and training. Additional ANOVA were run on the absolute reaction times before and after the respective retention intervals (including a 'before/after' factor). As these ANOVA essentially confirmed results for the percent improvements, they are not reported here.

Sleep parameters and subjective ratings were analyzed by ANOVA including the factors age, sleep/wake and training and, for subjective ratings also a factor 'time' (Retrieval 1 vs. 2). Analyses of sleep spindles included an additional factor for 'topography'. Generally, post-hoc t-tests were calculated if ANOVA revealed significant interactions. Greenhouse-Geisser correction of degrees of freedom was introduced where appropriate. A p-value < 0.05 was considered significant.

**Sleep and EEG Recordings.** Standard polysomnographic recordings were obtained using a portable amplifier (SOMNOscreen EEG 10-20, Somnomedics, Kist, Germany). Recordings were visually scored offline according to the criteria by Rechtschaffen & Kales (Rechtschaffen et al., 1968; Rechtschaffen et al., 1968). For each night, sleep onset, total sleep time, and the time as well as the percentage of total sleep time spent in the different sleep stages were determined. Sleep stages are wake, NonREM (REM – rapid eye movement) sleep stages 1, 2, 3, and 4, slow wave sleep (SWS, i.e. the sum of stage 3 and 4 sleep) and REM sleep. Sleep onset latency (i.e., the first occurrence of a period of stage 1 sleep followed by stage 2 sleep) was determined with reference to the time of lights off. Latencies of SWS and REM sleep were determined with reference to sleep onset.

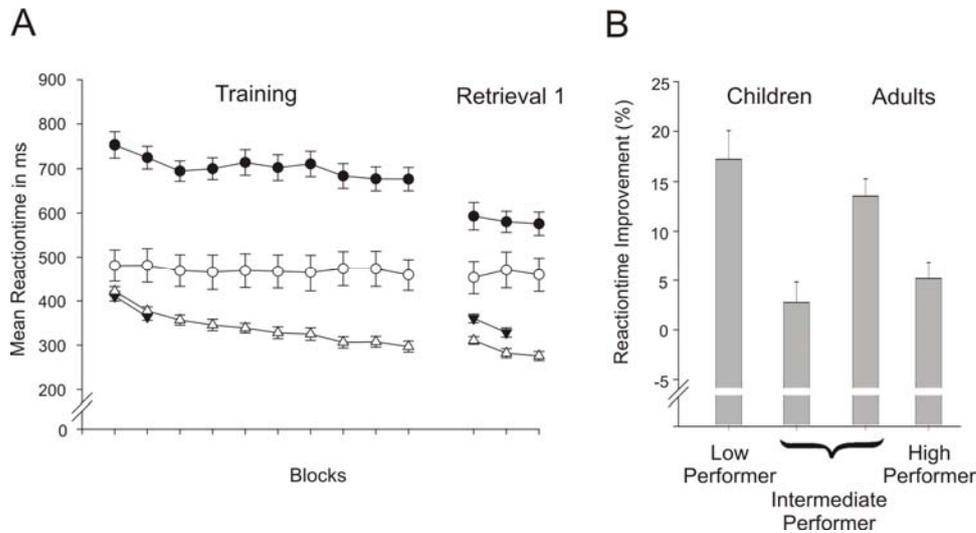
EEG spindles were identified automatically in NonREM sleep stage 2 and SWS separately at frontal, central and parietal sites (F3, F4, C3, C4, P3, P4) using a custom-made software tool (SpindleToolbox V.3) that was based on an algorithm adopted from previous studies (Mölle, Marshall, Gais, & Born, 2002). Briefly, first for each individual and recording channel the average power spectrum was calculated enabling the user to visually detect the individual peak of the spindle frequency band. Then, the root mean square (RMS) of the band-pass filtered signal in the range  $\pm 1.5$  Hz around the detected spindle peak of each 200 msec interval was calculated and the events were counted for which the RMS signal exceeded a constant threshold of 5  $\mu$ V for 0.5 – 3 sec. The mean number of spindles were calculated separately for frontal (F3, F4), central (C3, C4) and parietal (P3, P4) recording sites.

## Results

**Training Performance.** Performance continuously improved across the blocks of training on the motor sequence task in both children and adult groups ( $p < 0.001$  for main effect of 'block'), and also between the three training sessions the children with high amounts of training performed on 3 succeeding days ( $p < 0.01$ , for main effect of 'session', Figure 13A). Importantly, the four experimental groups differed distinctly according to the motor performance they reached at the end of training. As expected, at the end of training children with high amounts of training (3 times 10 blocks on 3 different days) showed faster reaction times than children with intermediate amounts of training (10 blocks of training on the day before the sleep/wake retention intervals,  $p < 0.001$ ); and adults with intermediate training showed faster reaction times than adults with low amounts of training (2 blocks on the day before the retention intervals,  $p < 0.001$ ;  $p < 0.001$ , for main effect of 'training'). Moreover,

adults were generally faster than children ( $p < 0.001$ , main effect of age). The extension of training from 10 to 30 blocks in children improved motor sequence performance to a greater extent than the extension from 2 to 10 blocks in adults ( $p = 0.014$ , for training  $\times$  age interaction). Accordingly, by manipulating the extent of training we were able to induce basically three different levels of motor performance reached during the learning phase: low-performing children, intermediate-performing children and adults, and high-performing adults.

**Motor Sequence Performance 30 Minutes after Training (Retrieval 1).** Reaction times at the end of extended training can be slowed due to fatigue developing during training. In order to obtain baseline measures for evaluating offline consolidation that are not contaminated by fatigue-related processes, subjects performed on three blocks (two blocks in adults with low amounts of training) of the button-box task 30 minutes after the end of training. Compared with performance at the end of training, motor sequence performance at this first retrieval was significantly enhanced ( $p < 0.001$ ). Percent improvements in motor performance across the 30-min interval (with performance at the end of training set to 100%) were significantly greater when performance levels at the end of training were relatively low, i.e., in children with 10 blocks of training and in adults with 2 blocks of training ( $p < 0.001$ , for main effect of training, see Figure 13B).

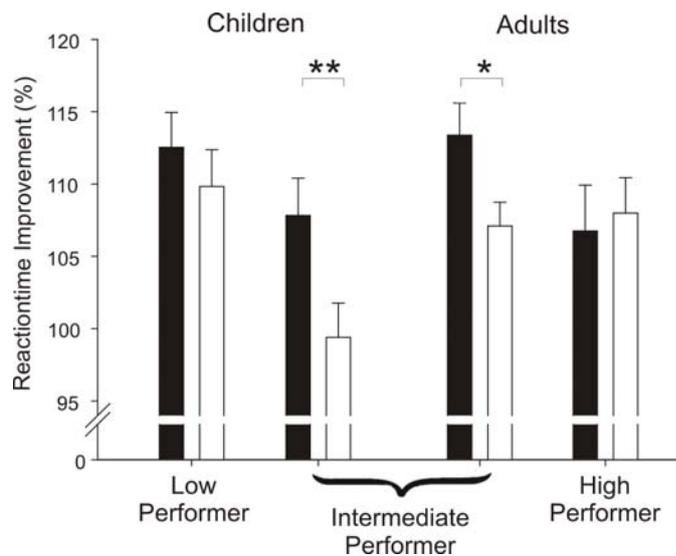


**Figure 13.** (A) Motor performance in low- and intermediate-performing children (filled and open circles, respectively) and intermediate- and high-performing adults (filled and open triangles, respectively) during training and at Retrieval 1. (B) Gains in motor performance at Retrieval 1, i.e., 30 minutes after training (in percent with performance at the end of training set to 100%), for low-, intermediate- and high-performing children and adults, respectively. Since there were no differences between the respective sleep and wake conditions ( $p > 0.23$ ) data are pooled across these conditions.

### Gains in Motor Sequence Performance across Sleep and Wake Retention Intervals.

Reaction times on the button-box task were again tested on three blocks after the ~120-min retention intervals filled with a nap or a comparable period of wakefulness (Retrieval 2). Generally motor performance further improved across the retention interval, with reference to performance at the first retrieval testing (Retrieval 1) before the retention interval ( $p < 0.001$ ). Independent of age, subjects who had slept during the retention interval showed greater performance gains than the subjects who had stayed awake ( $p = 0.017$ , for sleep/wake main effect, see Figure 14). However, the effect of sleep distinctly depended on the pre-sleep performance level ( $p = 0.048$ , for sleep/wake  $\times$  age  $\times$  training). In low-performing children (i.e., after 10 blocks of training), reaction time gains did not significantly differ between the sleep and wake retention interval (sleep:  $12.54 \pm 2.41$  %, wake:  $9.83 \pm 2.55$  %;  $p > 0.49$ ) whereas in intermediate-performing children (i.e., after three times 10 blocks of training) the performance gain was distinctly greater after retention sleep than wakefulness ( $7.83 \pm 2.58$  versus  $-0.60 \pm 2.36$  %;  $p = 0.006$ ). Complementary to this pattern in children, in adults performance gains across the retention interval did not significantly differ for the high-performing group (i.e., after 10 blocks of training; sleep:  $6.76 \pm 3.19$  %, wake:  $8.00 \pm 2.44$  %;  $p > 0.76$ ) whereas the gain in motor performance was significantly greater after retention

sleep than wakefulness in the intermediate-performing adults (i.e., after 2 blocks of training;  $13.38 \pm 2.22$  vs.  $7.10 \pm 1.64$  %,  $p = 0.019$ ). Thus, across age, sleep induced most robust gains in motor sequence performance when pre-sleep performance was at an intermediate level, whereas no sleep-associated gains in reaction time were obtained when pre-sleep performance was either very high or very low.



**Figure 14.** Gains in motor performance after retention sleep (black bars) and wakefulness (white bars) in low-, intermediate- and high-performing subjects indicated by the per cent difference in performance between the first and second retrieval (with performance at Retrieval 1 set to 100 %). \*\* -  $p < 0.01$ , \* -  $p < 0.05$ , for pairwise comparisons between sleep and wake conditions.

**Sleep, Spindles and Subjective Ratings.** Table 1 summarizes polysomnographical results. Compared with adults, children of the sleep groups spent less time awake and in NonREM sleep stage 1 and stage 2 ( $p < 0.001$ ), but more time in slow wave sleep (SWS;  $p < 0.001$ ). Children displayed a shorter SWS latency ( $p < 0.001$ ) than adults. There were no significant differences between the age groups for time in REM sleep or any other of the sleep parameters (for all comparisons  $p > 0.10$ ).

Analyses of EEG spindles revealed that the number of spindles was generally higher in adults than children ( $p = 0.023$ , for main effect of age), and also generally higher over the anterior than the posterior cortex ( $p < 0.001$ , for main effect of topography) with this topographical distribution being more evident in children than adults ( $p = 0.005$ , for age x topography). Spindle counts were significantly correlated with the absolute gain in reaction

time performance over the retention interval, but only in the intermediate performing adults who showed robust benefits from sleep after training (frontal:  $r = 0.50$ ,  $p < 0.08$ ; central:  $r = 0.52$ ,  $p < 0.07$ ; parietal:  $r = 0.56$ ,  $p < 0.05$ ) and not in the high-performing adults or in the children (all  $p > 0.12$ ).

Ratings indicated that subjects generally felt more tired before the retention interval (at the first retrieval) than after (i.e., at the second retrieval; children:  $p = 0.04$ ; adults:  $p = 0.005$ , for main effect of time) with this decrease in tiredness being more pronounced before retention sleep than retention wakefulness (children:  $p < 0.001$ ; adults:  $p < 0.001$ , for sleep/wake  $\times$  time). The amount of training did not affect tiredness (all  $p > 0.12$ ). Adults in the wake conditions in comparison to the sleep conditions indicated to be more motivated before and after the retention interval ( $p = 0.01$ , for sleep/wake main effect). However, rated motivation did not correlate with the gain in motor performance (all  $r < 0.17$ ,  $p > 0.32$ ) excluding a confounding impact of motivation. In children, rated motivation tended to increase after retention sleep and to decrease after retention wakefulness ( $p = 0.056$ , for sleep/wake  $\times$  time). Again, the amount of training did not affect rated motivation neither in children nor adults (all  $p > 0.18$ ).

**Table 4.** Sleep Data

	<b>Children Low Performer</b>	<b>Children Intermediate Performer</b>	<b>Adults Intermediate Performer</b>	<b>Adults High Performer</b>	<b>P -values</b>
Total Sleep Time (min)	66.6 ± 4.0	66.1 ± 2.9	70.71 ± 6.6	66.04 ± 7.6	< 0.001
Sleep onset (min)	20.7 ± 2.3	25.0 ± 2.6	26.06 ± 4.1	28.5 ± 5.3	n.s.
SWS latency (min)	8.00 ± 0.7	8.5 ± 0.9	29.1 ± 6.2	43.0 ± 6.4	< 0.001
REM latency (min)	63.9 ± 4.7	59.4 ± 3.5	68.9 ± 7.2	71.5 ± 5.2	n.s.
<b>Sleep stages - time in min</b>					
Wake	3.1 ± 0.7	3.1 ± 0.9	7.5 ± 1.1	12.1 ± 3.2	< 0.001
Stage 1	3.7 ± 0.4	4.3 ± 0.7	7.9 ± 0.9	7.5 ± 1.4	< 0.001
Stage 2	13.8 ± 1.9	14.5 ± 2.0	35.6 ± 3.9	33.7 ± 4.8	< 0.001
SWS	43.0 ± 2.9	40.5 ± 3.6	14.7 ± 2.8	8.3 ± 2.8	< 0.001
REM	2.9 ± 1.0	3.6 ± 0.8	4.5 ± 1.5	4.1 ± 2.1	n.s.
<b>Sleep stages - % of TST</b>					
Wake	4.6 ± 1.1	4.9 ± 1.6	11.8 ± 1.9	19.8 ± 4.4	< 0.001
Stage 1	5.6 ± 0.6	6.6 ± 1.1	12.8 ± 2.1	11.7 ± 1.7	< 0.001
Stage 2	20.1 ± 2.1	21.9 ± 2.6	49.5 ± 2.4	52.0 ± 4.7	< 0.001
SWS	65.6 ± 3.2	60.9 ± 3.9	20.2 ± 3.4	11.4 ± 4.4	< 0.001
REM	4.0 ± 1.5	5.6 ± 1.2	5.0 ± 1.7	4.5 ± 2.4	n.s.

Mean ( $\pm$  s.e.m.) total sleep time (TST), latency of SWS and REM sleep (in minutes, with reference to sleep onset) and time spent awake, in stage 1 sleep, stage 2 sleep, slow wave sleep (SWS) and rapid eye movement (REM) sleep in minutes and percentage of total sleep time. Right column indicates p-values for the main effect in the ANOVA. Differences in sleep parameters within the children or adults performing at the different levels were not significant.

## Discussion

Our data show that the level of performance reached after different amounts of training on a motor sequence task crucially affects whether this motor skill benefits from a subsequent nap, both in children and adults. Whereas our children after a standard amount of training did not show sleep-dependent gains in motor sequence performance, they did so after extensive training, indicating that children's low performance level can be one factor that prevented the development of sleep associated benefits for skill memory in previous studies (Wilhelm et al., 2008; Fischer et al., 2007; Prehn-Kristensen et al., 2009). Whereas our adults after a standard amount of training did not exhibit nap-related improvements in motor skill, they did so when pre-sleep training was reduced to only two blocks of practice. Because with this low amounts of training the adults' pre-sleep performance came close to the children's performance after extensive training, our findings overall suggest that, independent of the age, the benefitting

effect of sleep on motor skill is most robust with an intermediate level of pre-sleep performance.

Although considerable evidence has been accumulated that sleep supports the consolidation of skill memory, respective findings have been often questioned based on the presence of confounding factors, most importantly circadian factors and fatigue developing during learning (Cai et al., 2009; Rickard et al., 2008; Song et al., 2007). Specifically, it was argued that in studies comparing nocturnal sleep with daytime wakefulness, the behavioural expression of learning at the end of training in the evening is much lower than the actual learning performance due (i) to a circadian low in learning capability in the evening and (ii) due to fatigue gradually increasing in the course of training (Keisler et al., 2007). Here, we excluded these potential confounding factors (i) by investigating effects of sleep and waking on motor performance at the same circadian time, i.e., before and after a midday-nap or a parallel period of wakefulness (ii) by assessing pre-sleep performance level in a separate retrieval test (Retrieval 1) that took place 30 minutes after training had ended to allow recovery from fatigue. Our results confirmed in the presence of these controls that compared with waking the increase in motor sequence performance after a 2-hours retention interval was significantly greater when sleep occurred during this interval (Walker et al., 2003a).

Several studies in adults indicated an impact of the pre-sleep level of performance on sleep-dependent memory consolidation (Albouy et al., 2008; Hauptmann et al., 2005; Kuriyama et al., 2004) which led us to hypothesize that the lacking benefit from sleep for motor skill memories that was consistently found in children, resulted from the children's slower and less automated pre-sleep task performance (Fischer et al., 2007; Wilhelm et al., 2008; Prehn-Kristensen et al., 2009). Confirming this hypothesis we found that in children who received a three-fold increased amount of training on the motor sequence sleep indeed improved the motor skill. Reaction times in these children - although remaining still significantly lower - approximated those in adults who received minimum training of only two blocks on the sequence. As these two experimental groups, i.e., children after extended training and adults after restricted amounts of training, benefitted most from the nap, whereas the low-performing children and the high-performing adults after standard amount of training both showed no consistent benefit from the nap, our findings well agree with the view that sleep preferentially benefits motor memories at an intermediate performance level (Stickgold, 2009).

Thus, in order to directly profit from sleep motor skills in children need to be more intensely trained. Whether it is the mere strengthening itself of the skill representation or the

greater relevance attached to the skill after repeated training that enables the sleep-dependent skill improvement, is presently not clear (Wilhelm et al., 2011). Likewise, we can only speculate about the neurophysiological mechanisms underlying the consolidation of motor sequence memories at an improved but not at low pre-sleep performance level in children. In adults, patterns of brain activity distinctly change in the course of motor sequence learning. Activity in the prefrontal cortex, cerebellum and parietal cortex gradually decreases whereas activation in the striatum, primary motor cortex, supplementary motor areas and hippocampus increases (Willingham, 1998; Karni et al., 1998; Doyon et al., 2005; Albouy et al., 2008). It has been proposed that activation in hippocampal areas during learning determines to what extent a memory undergoes sleep-dependent consolidation, and this may also hold for sleep-dependent benefits in motor sequence learning (Diekelmann et al., 2009; Rauchs et al., 2011; Spencer et al., 2006). In fact, activation in this region predicted memory gains after sleep in a motor sequence learning task in adults (Albouy et al., 2008). Children's motor performance is distinctly slower and less automated compared to adults (Meulemans et al., 1998; Wilhelm et al., 2008), and this coincided with comparably less hippocampal activation during sequence learning in a (probabilistic) serial reaction time task (Thomas et al., 2004). Based on those findings we suppose that extended training on the motor sequence task in our study enhanced hippocampal recruitment in the children, to an extent where sleep leads to a distinct improvement in these motor sequence skills.

We found a significant correlation between the number of sleep spindles during NonREM sleep stage 2 and SWS and the sleep-associated gain in motor sequence performance in the adults whose motor skill improved by sleep, i.e., the group with restricted training. This finding confirms a number of previous studies indicating a robust link between sleep spindles and the sleep-dependent consolidation of motor memories (Rasch et al., 2009; Morin et al., 2008; Nishida et al., 2007; Tamaki, Matsuoka, Nittono, & Hori, 2008; Fogel et al., 2011). Spindles synchronize gamma band activity between different neocortical networks and in doing so, could be a mechanism enhancing the interlinkage between neocortical parts of the motor sequence representation (Ayoub, Mölle, Preissl, & Born, in prep). Surprisingly, this correlation between spindle counts and sleep related improvement in motor sequence performance was not obtained in the children who profited from sleep, i.e., the children with extended training. Although the difference in respective correlations between adults and children did not reach significance, this finding could point towards differences in memory processing during sleep between the age groups, requiring further examination.

Conceptually, our findings of a sleep-dependent improvement in motor sequence skill specifically with intermediate levels of pre-sleep performance may be explained on the basis of an interaction between explicit and implicit memory systems. Procedural sequence motor tasks like the button-box task of the present study comprise implicit and explicit components, both determining the final response speed (Schendan et al., 2003; Fletcher et al., 2005; Shanks et al., 1999; Sun et al., 2007; Willingham, 1998). Sleep has been found to preferentially facilitate the consolidation of explicit aspects of a task representation (Robertson et al., 2004; Spencer et al., 2006; Diekelmann et al., 2010). Importantly, whether explicit knowledge strengthened during sleep helps or hampers motor sequence performance strongly depends on the performance level. At an early stage of motor learning explicit knowledge may even deteriorate skill performance because both hippocampus-dependent explicit systems and cortico-striatal systems underlying implicit skill performance competitively interact (Willingham, 1998; Poldrack et al., 2001; Albouy et al., 2008). At a more advanced stage of performance, implicit aspects of the representations may be strong enough not to be essentially disturbed by sleep-dependent gains in explicit knowledge, enabling sleep to directly enhance implicit aspects of the task representation. Because children show a superior capacity for enhancing explicit aspects in memory during sleep (see Study 2), in the low-performing children with only weak implicit skill representations, any sleep-dependent gains in implicit motor performance might be nullified by competing interactions with explicit aspects that were strengthened during sleep. It appears that children's implicit skill memories only after extended training reach a level of strength and independence, which is comparable to that adults obtain with only a minimum of practice, and which allows for an unhampered emergence of a gain in motor skill after sleep. Such interaction between explicit and implicit task aspects very likely occurs during memory retrieval but may occur as well offline during consolidation.

## Conclusion and general discussion

The aim of the studies reported in the present thesis was to scrutinize sleep's role for memory consolidation in children, focusing on processes of motor memory consolidation. In contrast to adults sleep-dependent memory consolidation has been rarely studied in children. Due to high plasticity in the developing brain and great amounts of slow wave sleep (SWS) – both being demonstrated to be functionally related to memory consolidation during sleep in adults – together with little previous knowledge and schemes, children can serve as a model to investigate the basic mechanisms behind the processes of memory consolidation.

Together, the findings in the studies conducted in this thesis indicate that in children i) sleep improves the performance in declarative tasks but not the implicit performance in a motor sequence task - at least at early stages of learning (Study 1). In Study 2 it was found that ii) sleep benefits the generation of explicit knowledge from an implicitly learned motor task to a greater extent in children than in adults which iii) was correlated with high amounts of SWS and iv) coincided with greater hippocampal activation during retrieval. The results in Study 3 indicated that v) the lacking effect of sleep for implicit measures of a procedural task originates from children's low level of performance before sleep. The enhancement of motor performance before sleep intervals by prolonging the amount of training in children resulted in sleep-dependent gains in motor performance.

Retention of word- and card-pairs (Study 1) as well as explicit sequence knowledge within a motor task (Study 3) benefited from periods of post-learning sleep in children and adults. A common feature of these memory measures is that single elements needs to be bound together in these tasks, classically referred to as associative or relational memory. The formation of associative memories is well-known to critically rely on structures in the medial temporal lobe (MTL) system and the prefrontal cortex (PFC) in children as well as adults (Tubridy et al., 2010; Eichenbaum, 2004; Burgess et al., 2002; Thomas et al., 2004). In accordance, the anterior hippocampus was indeed highly activated during explicit sequence recall after retention sleep in the subjects' group showing highest explicit knowledge on the motor task (i.e., children's sleep-group, Study 3). These results are all the more striking as the hippocampus before puberty undergoes profound structural changes thereby being less selective and less specialized on a functional level (Gogtay et al., 2006; Ghetti et al., 2010; Menon et al., 2005). Possibly, it is this greater flexibility in the child's brain (Supekar et al., 2009) together with the specific sleep architecture that helps to qualitatively change a memory trace during the process of memory consolidation.

The previously mentioned data underline the importance of slow wave activity (SWA) in the consolidation of hippocampus-dependent memories. As expected, sleep in children was characterized by considerably greater amounts of time spent in SWS and a substantially higher power in SWA (0.5-4 Hz) than in adults in Studies 1-3. Peak levels of SWA are characteristic for children at the age of 10-12 years and reflect high synaptic density and synaptic efficacy as both factors boost neuronal synchronization (Buchmann et al., 2010; Kurth et al., 2010). The finding of SWA being significantly correlated with explicit sequence knowledge in adults (Study 2) is in line with a great number of previous studies conclusively demonstrating that SWS critically supports the consolidation of hippocampus-dependent memories in children and adults (Diekelmann et al., 2010; Backhaus et al., 2008). Moreover, the emergence of insight into a hidden rule of a task preferentially occurs within the first 3 hours of sleep which is characterized by great amounts of SWS (Yordanova et al., 2008). In Study 2, a correlation between SWA and explicit knowledge could not be calculated in the group of children because of perfect knowledge in 14 out of 16 children. Nevertheless, it can be speculated that the superior extraction of explicit knowledge during sleep in children is critically related to the peak level of SWA also in this age-group.

The findings that explicit knowledge on an implicitly learned motor task was greater after retention sleep compared to wakefulness is in line with general assumptions of the system consolidation theory (Frankland et al., 2005; Diekelmann et al., 2010). According to this theory, newly acquired memories are initially stored into a temporary buffer (i.e., the hippocampus in the declarative memory system). During sleep – and here mainly SWS – these memories are reactivated thereby becoming redistributed and integrated into the existing network of memories within the neocortex. In the process of system consolidation fresh memory representations are not only strengthened but also become reorganized and restructured. Wagner et al. (2004) reported that those subjects who had slept after implicitly learning a task with a hidden rule detected this rule with a twofold higher probability after the retention interval than subjects who had stayed awake after performing on the task (Wagner et al., 2004). In two further studies, sleep after performing on a probabilistic motor sequence task resulted in higher amounts of explicit knowledge on the sequence than wakefulness (Drosopoulos et al., 2010; Fischer et al., 2006). Although the data from Study 2 together with these previous studies confirm that sleep fosters qualitative changes of memories in the special case of transforming implicit to explicit memories there are first hints that sleep also produces qualitative changes in other domains. Sleep helps the extraction of a gist or a

schema from newly encoded memories - a process referring to changes of memory representations within the declarative system (Payne et al., 2008; Diekelmann et al., 2010).

Superior explicit sequence knowledge (Study 2) coinciding with lacking effects of sleep on implicit knowledge (Study 1) might result from a competitive interaction between these two aspects of a motor task. There is growing evidence that at initial stages of motor skill acquisition, explicit aspects may interfere with implicit task performance which resulted in decreased response speed (Jimenez et al., 1996; Willingham, 1998; Poldrack et al., 2001; Poldrack et al., 2003). Moreover, skill acquisition was accompanied by a competitive interference between respective striatal and prefronto-hippocampal systems (Poldrack et al., 2001; Poldrack et al., 2003; Schendan et al., 2003; Forkstam et al., 2005; Foerde et al., 2006). This competitive interaction is present at learning but can also extend to processes of memory consolidation and retrieval. In adults, the sleep-associated generation of explicit knowledge on a cognitive task interfered with the gain in implicit task performance (Wagner et al., 2004; Fischer et al., 2006). Subjects who had gained explicit knowledge of the sequence grammar in a SRTT after post-training sleep did not show the expected sleep-dependent speeding up of reaction times to grammatical cue positions (Fischer et al., 2006). Accordingly, explicit knowledge on the button-box task after retention sleep might have deteriorated performance speed at retrieval with this effect being even greater in children than adults due to their superior explicit knowledge. Thus, our findings on superior explicit knowledge after sleep in children in Study 2 can explain the lacking effect of sleep on implicit task performance which was found in Study 1.

Nevertheless, at later stages of learning, i.e., when the amount of training was elevated (Study 3), sleep benefited implicit task performance also in children. It seems as if implicit task performance only after extensive training reaches a level of strength at which it cannot be disturbed by explicit knowledge at retrieval. Interestingly, improving the amount of training in adults resulting in highly automated and rapid performance abolished the effects of sleep on motor performance. Thus, sleep supported the gain in motor performance at an intermediate performance level (i.e., in children with high amounts of training and adults with a restriction of training) but neither at a low- nor high performance level. These data are in line with previous theoretical suggestions arguing that sleep is most efficient at an intermediate performance level (Stickgold, 2009). To date, empirical data on the effects of the performance level at learning on sleep-dependent consolidation of motor memories is highly inconsistent with some studies showing effects of sleep only in high-performers (Albouy et al., 2008) while others reported sleep-dependent memory consolidation exclusively in low-

performers (Kuriyama et al., 2004; Song et al., 2007). Being incompatible at first glance, these discrepancies could be attributed to task-related factors containing the level of familiarity with the task as modulating factor. Performing on a highly complex task that is rather unfamiliar to subjects like the oculomotor task (Albouy et al., 2008) might result per se in relatively low levels of performance even in those subjects who perform highest in a study. In contrast, performing on a finger sequence tapping task (Kuriyama et al., 2004; Song et al., 2007) is highly familiar to subjects and thus performance rapidly reaches a highly automated and fast performance even in the low-performers of the study. Accordingly, high-performers in the oculomotor task can hardly be compared to high-performers in the finger sequence tapping task since the respective memory representation very likely differs. Instead, high-performers in the oculomotor task are possibly at the same level as low-performers in a finger-tapping task, i.e. at an intermediate level which would be completely in line with our data in Study 3. Importantly, these results have major implications for future studies focusing on the effects of sleep on motor memory consolidation. Several factors, namely the difficulty of a task, familiarity with a task, previous knowledge or the length of training can impact performance level at learning thereby determining whether sleep benefits motor memory consolidation or not.

Our findings on implicit and explicit measures of motor memory consolidation during sleep can be interpreted on the background of the “Radical Plasticity Thesis” formulated by Cleeremans (2008). According to this model a memory trace after initially being available as implicit cognition becomes explicit in the course of learning. With even more training the brain switches to an automatized mode of task performance at which cognitive control is no longer needed because this memory has proven to be relevant and adaptive in the way it exists. On the background of this model, there are two possible ways how sleep could benefit performance on a motor task. First, sleep might help switching from one to the next quality of a memory representation. Specifically, in low-performing children where the neuronal representation of the motor task is implicit during learning sleep might support the transfer to an explicit mode at recall (in line with the results in Study 2) whereas the automation as measured by reaction-times is not affected at this stage (see Study 1 and the low-performers in Study 3). After intense training the neuronal representation of the motor task becomes explicit and subsequent sleep might help switching to the state of automaticity which was observed by significant gains in reaction-times (observable in children and adults performing at an intermediate level in Study 2). Adults need less training to reach the state of explicit representation because of their extensive previous knowledge. After additional training on the

motor task in adults, performance becomes highly automated already before retention sleep thereby reducing the need for sleep to benefit motor learning. In contrast to sleeps' impact on switching from one to the next quality of a memory representation, during wakefulness performance might be further processed in the current state.

Alternatively, sleep might exclusively benefit the extraction of explicit knowledge at all stages of motor learning but whether explicit knowledge helps or hampers motor performance strongly depends on the performance level. As previously mentioned, at an early stage of motor learning explicit knowledge leads to a deterioration of performance (Willingham, 1998) whereas at the later stages of learning, explicit knowledge on a motor task can improve task performance (Dienes et al., 1999; Seger, 1994). Therefore, children at an early stage of learning (i.e., low performers) benefit from sleep in explicit knowledge (Study 2) which competitively interacts with motor performance (Study 1 and 3 – low performers). This is in contrast to adults as well as intermediate-performing children where explicit knowledge helps implicit task performance (Study 3). Accordingly, if sleep-dependent gains in motor performance are found in a study, it might reflect the successful extraction of explicit knowledge helping motor performance rather than the direct improvement of motor performance. In adults, who automatically perform on the motor task sleep either does no longer support the emergence of explicit knowledge, or explicit knowledge - although being still generated during sleep - does not further help implicit knowledge since motor sequence performance already reaches its individual limit of motor abilities.

Future studies need to finally answer the new questions that emerge on the background of the presented data with only some of these being referred. For example, in a future study it could be asked whether sleep benefits explicit aspects within a motor task at all stages of motor learning similarly or just at the beginning. Therefore, the emergence of explicit knowledge after sleep should be tested at very low, intermediate and high performance levels during learning. Peak levels of SWA can be found at around 12 years whereas SWA is much lower before and thereafter (Buchmann et al., 2010; Campbell et al., 2009). To further elucidate the critical role of SWA for the transition of implicit to explicit knowledge, children and adolescents at different ages (i.e., 8-10; 12-14; 16-18) need to be tested. Concurrent with the age-dependent variation in the amount of SWA one would expect an inverted U-shaped function with the sleep-dependent gain in explicit knowledge being highest in the group of 12-14 years old subjects. Another aspect to be addressed in the future are long-term effects of sleep in children's memory consolidation. Although sleep did not affect implicit task

performance in children at low levels of performance here, it might help motor performance at the long-term. Like the birds in the study by Deregnaucourt (Deregnaucourt et al., 2005), those children who show the greatest deterioration of motor performance after sleep might perform best at a later test. For this purpose, implicit performance in future studies should not only be tested in the morning after sleep but also in the evening of the same day and again after a longer delay. Finally, future fMRI studies should i) further explore the brain regions that are involved in the process of memory consolidation during sleep in children and ii) elucidate the neuronal prerequisites for the occurrence of sleep-dependent gains in performance. For example, fMRI studies could be performed to specify the neural underpinnings of sleep-dependent memory consolidation by testing task-related brain activity in children in the beginning of learning and after additional sessions of training.

## Abstract

Studies in adults have extensively shown that sleep supports the consolidation of memories. Children sleep more and deeper, with distinctly greater amounts of slow wave sleep (SWS). Although SWS is mechanistically implicated in memory consolidation, the effect of sleep on memory consolidation in children has so far been rarely investigated. In three studies, we tested the influence of sleep on declarative and procedural memory consolidation in children, presenting them learning tasks before retention intervals filled with sleep or wakefulness, and subsequently tested retrieval. Study 1, performed with 6-8 years old children revealed a clear improving effect of post-learning sleep on consolidation of declarative memories (word pairs), but we found no effect on the consolidation of procedural memories (finger sequence tapping task). We hypothesized that this lack of any sleep-dependent gain in motor skill memory is caused by the preferential consolidation of explicit over implicit aspects of the motor task such that after retention sleep explicit knowledge about the task starts to interfere with implicit motor regulation and slows task performance. Thus, in Study 2 children (8-11 years) and adults (20-35 years) were trained on a SRTT under implicit conditions (without explicit knowledge about the underlying sequence), and their explicit knowledge about the sequence was tested after retention sleep or wakefulness. Sleep enhanced explicit knowledge in both age groups, but to an overwhelming extent in children. Explicit knowledge after sleep was associated with greater amounts of slow wave activity (SWA) as well as stronger hippocampal activation during sequence recall, indicating that superior explicit knowledge was caused by the reactivation of hippocampus-dependent task aspects during sleep. Previous studies in adults showed that the level of motor performance critically determines whether sleep benefits memory consolidation or not, with intermediate levels possibly being most efficient in this context. We therefore tested in Study 3 whether sleep benefits procedural motor memories in children (4-6 years) if training is intensified to achieve an intermediate level of performance. In fact, a daytime nap in comparison to wakefulness significantly enhanced motor performance in children only after extensive training of the task. In control children with less extensive training, sleep did not benefit implicit knowledge on a motor task. Taken together, our work suggests a preferential benefit of explicit over implicit memories from sleep in children, possibly related to their greater amounts of SWS. Accordingly, the children's benefit from sleep is comparable to that in adults for declarative (i.e. explicit) memories, whereas benefits for procedural skills depend on the pre-sleep training level, occurring only at intermediate pre-sleep performance level.

## Zusammenfassung

Studien an Erwachsenen haben wiederholt gezeigt, dass Schlaf die Konsolidierung neugelernter Gedächtnisinhalte unterstützt. Der kindliche Schlaf dauert nicht nur länger sondern beinhaltet auch einen wesentlich größeren Anteil am langsamwelligen Deltaschlaf als der Schlaf eines Erwachsenen. Obwohl Deltaschlaf maßgeblich an Prozessen der schlafbezogenen Gedächtnisbildung beteiligt ist, wurde die Rolle des Schlafes für die Gedächtnisbildung bei Kindern bislang kaum untersucht. In der vorliegenden Doktorarbeit wurde im Rahmen von drei experimentellen Studien die schlafbezogene Konsolidierung prozeduraler und deklarativer Gedächtnisinhalte näher beleuchtet, indem entsprechendes Material vor einem Schlaf – bzw. Wachintervall gelernt und anschließend getestet wurde. Die erste Studie ergab einen positiven Effekt eines Schlaf- im Vergleich zu einem Wachintervall nach dem Lernen für die Konsolidierung deklarativer Inhalte (Wortpaare) allerdings wurde kein Effekt für die prozedurale Aufgabe (Fingersequenzaufgabe) gefunden. Es wurde daraufhin vermutet, dass das Fehlen des schlafbedingten Zuwachses in der motorischen Leistung (d.h. implizites Wissen) bedingt ist durch die vorrangige Konsolidierung expliziter Aspekte einer motorischen Aufgabe, wobei möglicherweise nach einem Schlafintervall verstärktes explizites Wissen über die Aufgabe mit der impliziten motorischen Ausführung der Aufgabe interferiert. Daher wurden in einer weiteren Studie Kinder (8-11 Jahre) und Erwachsene (20-35 Jahre) in einer Motorsequenzaufgabe (ohne explizites Wissen über die zu lernende Sequenz) trainiert. Explizites Wissen über die Sequenz wurde nach einem Schlaf- oder Wachintervall getestet. Schlaf verbesserte das explizite Wissen in beiden Altersgruppen, allerdings in weitaus größerem Maße bei Kindern. Das explizite Wissen nach dem Schlaf war assoziiert mit der Menge an Deltaschlaf und mit einer stärkeren hippocampalen Aktivierung während der Testung des expliziten Wissens. Auf Grundlage dieser Befunde kann vermutet werden, dass das ausgeprägte explizite Wissen über die gelernte motorische Fingersequenz durch die Reaktivierung vorrangig hippocampus-abhängiger Aufgabenaspekte bedingt wurde. Vorangegangene Studien an Erwachsenen legen nahe, dass das Niveau der motorischen Leistung beim Lernen ein entscheidender modulierender Faktor ist, der beeinflusst, ob Schlaf die Konsolidierung dieser Gedächtnisspur unterstützt oder nicht, wobei vor allem ein mittleres Leistungsniveau als optimal in diesem Zusammenhang beurteilt wurde. Daher wurde in der dritten Studie untersucht, ob Schlaf bei Kindern (4-6 Jahre) den Zuwachs der motorischen Gedächtnisleistung dann unterstützt, wenn das Training intensiviert wird. Tatsächlich verbesserte Schlaf nach dem Lernen einer motorischen Aufgabe die implizite Leistung, wenn

das Niveau vor einem Retentionsintervall durch extensives Training gesteigert wurde, jedoch nicht bei einer „Standardmenge“ an Training. Zusammenfassend zeigen die vorgestellten Studien, dass der deltaschlafreiche Schlaf bei Kindern vorrangig explizite Aspekte einer motorischen Aufgabe unterstützt während der schlafabhängige Zuwachs in der motorischen Leistung nur bei einem mittleren Leistungsniveau beim Lernen sichtbar ist.

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## **Appendix**

Die der vorliegenden Arbeit zugrunde liegenden Studien wurden von der Ethikkommission der Universität zu Lübeck genehmigt (Aktenzeichen: 07-031, Genehmigung am 21.05.2007; Aktenzeichen: 09-089, Genehmigung am 13.07.2009).

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