SLEEP'S ROLE ON EPISODIC MEMORY CONSOLIDATION

IN ADULTS AND CHILDREN

Dissertation

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von

Jing-Yi Wang

aus Shijiazhuang, Hebei, Volksrepublik China

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Dekan der Math.-Nat. Fakultät: Prof. Dr. W. Rosenstiel

Dekan der Medizinischen Fakultät: Prof. Dr. I. B. Autenrieth

1. Berichterstatter: Prof. Dr. Jan Born

2. Berichterstatter: Prof. Dr. Steffen Gais

Prüfungskommission: Prof. Manfred Hallschmid

        Prof. Dr. Steffen Gais

        Prof. Christoph Braun
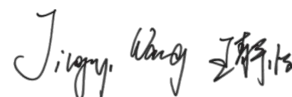
        Prof. Caterina Gawrilow

Declaration:

I hereby declare that I have produced the work entitled "**Sleep's Role on Episodic Memory Consolidation in Adults and Children**", submitted for the award of a doctorate, on my own (without external help), have used only the sources and aids indicated and have marked passages included from other works, whether verbatim or in content, as such. I swear upon oath that these statements are true and that I have not concealed anything. I am aware that making a false declaration under oath is punishable by a term of imprisonment of up to three years or by a fine.

Tübingen, the December 5, 2016 ....

Date   Signature

To my beloved parents – Hui Jiao and Xuewei Wang,

Grandfather – Jin Wang,

and

Frederik D. Weber

致我的父母：焦惠和王学伟

爷爷王金，以及

爱人王敬德

Content

Abbreviations

ANCOVA – Analysis of Covariance

ANOVA - Analysis of variance

EEG – Electroencephalography

HPC – Hippocampus

LTD – Long-term depression

LTP – Long-term potentiation

MTL – Medial temporal lobe

PFC – Prefrontal cortex

REM – Rapid eye movement

SEM – Standard error of the mean

SOs – Slow oscillations

SWA – Slow-wave activity

SW-Rs – Sharp wave ripples

SWS – Slow-wave sleep

WWWhen – What-Where-When paradigm

WWWhich – What-Where-Which paradigm

List of publications in the cumulative thesis

Weber, FD.*, **Wang, JY.***, Born, J. & Inostroza, M. (2014). Sleep benefits in parallel implicit and explicit measures of episodic memory. *Learning & Memory*. 2014. 21: 190-198 (*equal contribution)

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Wang, JY., Weber, FD., Zinke, K., Noack, H., & Born, J. Effects of sleep on word-pair memory in children – separating item and source memory aspects. (Submitted)

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Summary

Episodic memory is an essential cognitive function to support our everyday life. It depends on the hippocampus to bind the experienced events into their spatiotemporal contexts, i.e. giving us information about what has happened, where and when. Sleep is vital for declarative memory. Broad evidence suggests sleep as an optimal state to transfer a hippocampus-dependent memory from a temporary short-term representation into its stable neocortical long-term storage, i.e. its consolidation. This sleep-mediated consolidation process is thought to happen especially during deeper slow-wave sleep, i.e. a sleep stage most abundant in children before puberty. It is unclear how sleep consolidates particularly episodic memory, and how sleep-mediated consolidation changes during development. This dissertation aimed to establish new behavioral paradigms and study sleep's effect on episodic memory in adults and children. We hypothesized that sleep benefits specifically the consolidation of episodic aspects of memory, especially in children.

To explore sleep's effect on episodic memory consolidation in human adults (18-37 years) and children (8-12 years) we established a new episodic task to assess "What-Where-When" memory by explicit (oral report) and implicit (eye-tracking) measures. Additionally, we changed a word-pair learning task to an item–source paradigm, in which word pairs (items) were learned in temporal contexts (source) separated by two lists. This task allowed the assessment of episodic aspects of word-pair learning, i.e. the binding of item and source memory. Using the same tasks, children and adults encoded two episodes and learned two lists of word pairs, with each episode and list being separated by an hour. Memory was then tested on short-term (1-hour delay) prior to sleep as well as after long-term retention (~10-h delay) with either a night of sleep or a day of wakefulness. In adults, explicit and implicit measures of episodic memory were positively associated with each other, and both benefitted from sleep, thus linking this sleep benefit to previous rodent studies and opening the possibility to apply this paradigm to a broader age range (study I).

Comparing adults with children, both ages showed a two-fold benefit for episodic memory after sleep than after wakefulness, even though children had superior amounts of slow-wave sleep. Surprisingly, children started at a much lower episodic memory level on the short-term before sleep than adults, suggesting children had less capacity to encode or retain episodic memories. However, children did not forget the episodes any further over the any long-term retention interval as the adults did, suggesting the consolidation of episodic memory in children to be more efficient, and unrelated to sleep (study II).

Replicating previous studies, we also found that children benefit from sleep for the explicitly learned word-pair memory over sleep. Unlike the weak temporal memory effect in the episodic task, we found a benefit from sleep for the temporal context memory in the word-pair learning task. Although also here the children's capacity for episodic binding was reduced shortly after encoding (1-hour recall) as compared to adults, unlike in the episodic task, the children seemed rather to "unbind" the word-pair and temporal context memory over sleep. Moreover, children maintained semantic memory for the word pairs better on short-term, but, unlike adults, they showed forgetting over sleep. This suggests altered forgetting curves over sleep for episodic *vs.* semantic based memory (study III). Across studies, episodic aspects were in part positively correlated with sleep spindles (a hallmark feature of sleep) and slow-wave sleep, while semantic aspects correlated negatively with sleep spindles, suggesting different preferred roles for sleep spindles and slow-wave sleep in episodic and semantic memory consolidation. Notably, sleep more effectively consolidated the kind of memory that each age group retained worse on the short-term, e.g. the still developing episodic memory in children.

Taken together, this thesis supports the hypothesis that sleep benefits the consolidation of hippocampus-dependent memory for episodes and their spatial and temporal contexts. Children's lower capacity for episodic memories might be compensated by more effective consolidation mechanisms. The finding that deeper sleep in children might also favor unbinding of episodic memories (i.e. decontextualization) should be scrutinized in future studies.

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Zusammenfassung

Das episodische Gedächtnis ist eine kognitive Funktion, die unverzichtbar ist für unser tägliches Leben. Es benötigt den Hippocampus, um die erfahrenen Ereignisse in ihren örtlichen und zeitlichen Kontext zu binden, d.h. es gibt uns Informationen darüber, was passiert ist, wo und auch wann. Schlaf ist unerlässlich für das deklarative Gedächtnis. Umfassende wissenschaftliche Untersuchungen deuten auf Schlaf als einen optimalen Zustand hin, um eine vom Hippocampus abhängige Gedächtnisspur von einer temporären Repräsentation im Kurzzeitgedächtnis hin zu einer stabil, gespeicherten Langzeitgedächtnisspur im Neocortex zu transferieren, d.h. ihre Konsolidierung. Es wird angenommen, dass dieser von Schlaf vermittelte Konsolidierungsprozess besonders während des tieferen langsamwelligen Schlafes (sog. Deltaschlaf oder engl. „slow-wave sleep") stattfindet, d. h. in Schlafstadien, welche am häufigsten im Schlaf von Kindern vor der Pubertät vorkommen. Es ist unklar, wie Schlaf im Besonderen das episodische Gedächtnis konsolidiert, und auch wie die schlafvermittelte Konsolidierung sich während der körperlichen und geistigen Entwicklung verändert. Diese Dissertation hat zum Ziel neue Verhaltensparadigmen zu etablieren und den Effekt von Schlaf auf das episodische Gedächtnis in Erwachsenen und Kindern zu erforschen. Um den Effekt von Schlaf auf die Konsolidierung von episodischem Gedächtnis bei menschlichen Erwachsenen (18-37 Jahre) und Kindern (8-12 Jahre) zu explorieren, haben wir eine neue episodische Aufgabe etabliert, um das „Was-Wann-Wo"-Gedächtnis (engl. „What-Where-When" memory) mittels expliziten (mündliche Abfrage) und impliziten (Eye-tracking) Messmethoden zu bestimmen. Zusätzlich haben wir eine Wortpaarlernaufgabe abgewandelt, bei der Wortpaare als Gedächtniselemente und zwei Wortpaarlisten als zeitlichen Kontext für den Gedächtnisursprung dienten (engl. „item-source"-Paradigma). Diese Aufgabe ermöglichte das Bestimmen von episodischen Gedächtnisaspekten des Wortpaarlernens, d.h. die Gedächtnisverbindungen zwischen Gedächtniselementen und ihrem zeitlichen Gedächtnisursprung. Kinder und Erwachsene enkodierten unter Verwendung derselben Aufgaben zwei Episoden und lernten zwei Wortpaarlisten, jede jeweils zeitlich durch eine Stunde getrennt. Das Gedächtnis wurde dann darauf getestet, wie viel nach einem kurzen Zeitintervall (1 Stunde Verzögerung) vor dem Schlaf, oder nach einem langen Zeitintervall (~10 Stunden Verzögerung) mit entweder einem Nachtschlaf oder einer Wachheit am Tag, behalten wurde. In Erwachsenen waren die expliziten und impliziten Messgrößen des episodischen Gedächtnisses positiv miteinander assoziiert und beide profitierten vom Schlaf. Dabei lässt sich wurde der hier aufgezeigte Nutzen von Schlaf in unseren Ergebnissen mit denen von vorherigen Studien bei Nagetieren verbinden und eröffnete uns auch die Möglichkeit diese Paradigma auf eine breitere Altersspanne anzuwenden (Studie I). Im Vergleich von Erwachsenen mit Kindern zeigten beide Altersgruppen ein doppelt so gutes

episodisches Gedächtnis nach dem Schlaf als nach der Wachheit über den Tag, und das obwohl die Kinder weitaus höhere Anteile an langwelligem Schlaf aufzeigten. Überraschenderweise starteten die Kinder auf einem viel niedrigeren Niveau als die Erwachsenen für episodisches Gedächtnis wenn der Abruf nach dem kurzen Zeitintervall vor dem Schlaf erfolgte. Das deutet darauf hin, dass die Kinder eine niedrigere Kapazität hatten die Episoden zu encodieren oder diese im Gedächtnis zu behalten. Jedoch vergaßen die Kinder die Episoden über die den langen Zeitintervall nicht noch weiter sowie die Erwachsenen, was auch wiederum darauf hindeutet, dass die Konsolidierung vom episodischen Gedächtnis effizienter in Kindern und auch unabhängig von Schlaf zu sein scheint (Studie II). Auch beim Replizieren von vorigen Studien konnten wir ebenso einen Vorteil von Schlaf für das explizit gelernte Wortpaar-Gedächtnis in Kindern über das gemessene Schlafinterval feststellen. Im Gegensatz zu dem schwachen Effekt für das Zeitgedächtnis in der episodischen Gedächtnisaufgabe, fanden wir, dass Schlaf auch das Gedächtnis des zeitlichen Kontexts während der Wortpaarlernaufgabe unterstützte. Obwohl auch hier die Kapazität für episodische Gedächtnisverbindungen kurz nach dem encodieren (Abruf nach 1 Stunde) im Vergleich zu den Erwachsenen schon reduziert war, schienen die Kinder, im Gegensatz zu der episodischen Gedächtnisaufgabe, ihre encodierten Gedächtnisverbindungen zwischen den Wortpaaren und deren zeitlichen Kontext über Schlaf hinweg eher wieder zu lösen. Zudem behielten die Kinder semantische Gedächtnisinhalte für die Wortpaare besser über den kurzen Zeitintervall, aber im Gegensatz zu den Erwachsenen, zeigten die Kinder auch ein Vergessen dieser Inhalte über den Schlafintervall hinweg. Dies legt unterschiedliche Vergessenskurven über Intervalle mit Schlaf für das episodisch- vs. das semantisch, gestützte Gedächtnis nahe (Studie III). Über alle Studien hinweg korrelierten die episodischen Gedächtnisaspekte zum Teil positiv mit Schlafspindeln (ein kennzeichnendes Merkmal für Schlaf) und langsamwelligem Schlaf, wohingegen semantische Gedächtnisaspekte negativ mit Schlafspindeln korrelierten. Dies deutet auf eine unterschiedliche Präferenz für die Rolle von Schlafspindeln und von langsamwelligem Schlaf in der Konsolidierung vom episodischen und semantischen Gedächtnis hin. Beachtenswert ist auch, dass Schlaf gerade die Art von Gedächtnis effektiver konsolidierte, welche jede Altersgruppe über den kurzen Zeitintervall hinweg schlechter behielt, was z.B. bei den Kindern das sich immer noch entwickelnde episodische Gedächtnis war. Zusammengefasst unterstützt diese Dissertation die Hypothese, dass Schlaf die Konsolidierung des vom Hippocampus abhängigen Gedächtnisses für Episoden und deren örtlichen und zeitlichen Kontext unterstützt. Die geringere Kapazität für episodisches Gedächtnis bei Kindern könnte durch deren effektivere Konsolidierungsmechanismen kompensiert sein. Der Befund, dass der tiefere Schlaf in Kinder auch das Lösen von episodischen Gedächtnisverbindungen begünstigen kann (d.h. eine Dekontextualisierung der Gedächtnisinhalte), sollte in zukünftigen Studien nochmals genauer überprüft werden.

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Synopsis

Introduction

1. Memory in general

Memory is a fascinating cognitive function of the brain. To form a memory, one needs to encode, store, and subsequently recall information at later time points. Having memories, we can therefore form our self-identity, acquire and store new knowledge or skills, and even accordingly plan and make decisions for the future. Memory is also a key for learning processes, through which we gain knowledge of the world and modify our subsequent behavior (Mastin, 2010). During learning (intentionally or incidentally), neurons at specific brain areas fire together to build up connections (Hebb, 1949). "Cells that fire together, wire together" well described the classic Hebbian's theory of learning (Lowel & Singer, 1992). When those connections are consolidated and later can be retrieved, memories are formed.

1.1 The process of memory formation

The process to form a memory refers to encoding, consolidation, storage and retrieval. *Encoding* converts the perceived items into a construct that stores as memory. At the neuronal level, encoding refers to synaptic long-term potentiation (LTP) or long-term depression (LTD), which are the primary form of learning-dependent synaptic plasticity (Poo et al., 2016). Perception and attention are involved in the encoding process; also emotion influences memory encoding by raising arousals (Sharot & Phelps, 2004). *Consolidation* is the process of stabilizing a memory trace after the initial acquisition (Yadin Dudai, 2004). Consolidation is considered to consist of two levels of processes: "synaptic consolidation" and "system consolidation". *Synaptic consolidation*, happening within a few hours after encoding, refers to the remodeling of synapse and spines of neurons that relate to memory representations and gradually lead to enduring changes (Kandel, 2001). *System consolidation* involves a continuous

process that pushes newly encoded memory representations to redistribute into long-term storage over hours to years (Dudai, 2004; Frankland & Bontempi, 2005). The standard consolidation theory hypothesized that hippocampus and related structures in the MTL serve as a temporary store of newly encoded memory traces. Over time, by reactivation of hippocampal memory networks, new information gradually integrates into the neocortex to become long-term memories (Marr, 1971). *Retrieval* of memory refers to re-accessing events or information from the past, which have been previously encoded and stored in the brain.

After successful encoding, a failed consolidation or retrieval leads to memory forgetting, which may indicate a memory is either physically unavailable (a memory gets lost), or the memory is temporally inaccessible (Hardt, Nader, & Nadel, 2013). Although we will not remember every detail of our daily life (e.g., what did you eat for lunch 100 days before), there are memories we always keep in mind, for example, our family name (but see a special kind of humans who seem to remember every single thing that they experienced by just giving temporal cues from Parker, Cahill, & McGaugh, 2006). Interference theory assumes that forgetting is due to the competition of newly encoded information and previous stored old memory (Tomlinson, Huber, Rieth, & Davelaar, 2009). *Proactive interference* is "forgetting due to interference from the traces of events or learning that occurred prior to the materials to be remembered" (Still, 1969), while *retroactive interference* happens when newly learned information interferes with the recall of previously learned information (Wohldmann, Healy, & Bourne, 2008).

The memories that have been stored successfully into the long-term storage are not always accurate (Loftus, 2005). Apart from being interfered or forgotten, the stability of the stored memories could also undergo a process of *Reconsolidation*, which assumes that consolidated memories may enter a labile state after the retrieval (Forcato et al., 2007; Forcato, Rodriguez, Pedreira, & Maldonado, 2010). Reconsolidation is not merely a paradoxical process to erase already previously acquired memory, but rather suggested to be "a form of new learning" that during retrieval, the ostensibly consolidated memories get "updated" with current new information (S. H. Wang & Morris, 2010).

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1.2 Memory types

According to the Atkinson-Shiffrin model (Atkinson & Shiffrin, 1968), there are mainly three

kinds of memories that are categorized with the lifespan (or stages) of the memory: *Sensory*

memory holds sensory information less than one second after an item is perceived. It includes

Iconic memory (Sperling, 1960), Echoic memory (Neisser, 1967) and Haptic memory (Bliss,

Crane, Mansfield, & Townsend, 1966), which three represent for the visual, auditory and tactile

sensory memories. *Short-term memory* allows recall after period of several seconds to a minute

without rehearsal. Working memory has a short-term buffer, but it is not exactly short-term

memory (Diamond, 2013).

This thesis, however, focuses mainly on *Long-term memory*, which stores information over

long periods, i.e. from hours after encoding but potentially lasting up to years. Additionally,

depending on whether a memory can be accessed consciously or not, long-term memories are

categorized as declarative memory (i.e. explicit memory) and non-declarative memory (i.e.

implicit, in the traditional view). Although a new model was proposed according to the

processing mode rather than conscious access (Henke, 2010), for simplicity, this thesis is going

to discuss different declarative memories, mainly episodic memory, in the following sections

according to the traditional (explicit) model.

2. Episodic memory and related brain structures

2.1 What is episodic memory?

Episodic memory is a part of the declarative memory system. It describes the ability to

remember specific events or episodes that happened in our personal past, in contrast to the

ability to simply know the knowledge or facts about the world (Salwiczek, Watanabe, &

Clayton, 2010). According to the definition from Endel Tulving, episodic memory is "an

information processing system that receives and stores information about temporally dated

episodes or events, and about temporal-spatial relations among these events." (Tulving, 1972)

Episodic memory is crucial to guide our daily activates such as remembering where you need to

go to fetch your parked car after work, or recollecting what you ate recently in which restaurant that could cause you sickness right now.

Episodic memory used to be considered as a human-unique ability because recollection is assumed to be essential for the retrieval process (Tulving, 1983). However, evidence in animals gradually accumulates to reveal that many of them also seem to have episodic-like memory, though it is hard to detect the autonoetic consciousness, which is supposed to be essential for human-like episodic memory. Therefore similar behavior mimicking important aspects of episodic in animals have been identified as "episodic-like" memory and can, for example, be found in primates (Beran et al., 2016) also see review from (Schwartz & Evans, 2001), rats (Inostroza, Binder, & Born, 2013), mice (Fellini & Morellini, 2013), birds (N. Clayton, 1998), and even in invertebrates (Jozet-Alves, Bertin, & Clayton, 2013).

The episodic memory, when the sense of self is involved, is called autobiographic memory (Nelson & Fivush, 2004). It forms our personal identity since one can locate him-/herself into a past time that he or she must be able to identify him- or herself in nowadays (Klein & Nichols, 2012).

2.2 Neural basis of episodic memory

2.2.1 Encoding

Medial temporal lobe (MTL) and multiple neocortical areas are involved in episodic memory encoding. Hippocampus (HPC), a core structure of MTL, is essential for the formation of new episodic memory (Eichenbaum, Sauvage, Fortin, Komorowski, & Lipton, 2012). Patients with injured HPC (for example, the famous patient H.M., whose bilateral hippocampus were removed for the reason of severe epilepsy) cannot form new long-term memory for events (for example, who have you met yesterday at school).

At encoding, HPC obligatorily receives inputs from entorhinal cortex, which in turn accept inputs from perirhinal cortex and parahippocampal cortex. HPC then is thought to integrate the parts and features about object representations from the perirhinal cortex and view-specific scene representations from the parahippocampal cortex into a spatiotemporal frame

(Moscovitch, Cabeza, Winocur, & Nadel, 2016; Nadel & Peterson, 2013). Within HPC, by receiving signals from the posterior neocortex, the posterior end of HPC captures detailed local spatiotemporal aspects of an experienced event, while the anterior end of HPC captures global aspects of an event, which is based on interactions with the anterior neocortex (Moscovitch et al., 2016; Strange, Witter, Lein, & Moser, 2014). Apart from this, prefrontal cortex (PFC) and related brain structures participate in episodic encoding by a top-down modulation (Nolde, Johnson, & Raye, 1998). Neuroimaging evidence suggests that areas in the PFC, mainly ventromedial PFC (vmPFC) and ventrolateral PFC (vlPFC), aid the organization of information (Ranganath & Knight, 2002) and also underlie the execution of semantic strategies which enhance encoding (Gabrieli, Poldrack, & Desmond, 1998), e.g. thinking about the meaning of the study material or rehearsing it in working memory. Also, attention and emotion also influence episodic memory encoding. Attention, another hallmark of autonoetic consciousness, determines mostly how well different components of an episode are encoded (Guerin, Robbins, Gilmore, & Schacter, 2012). This process is supported by ventral partial cortex (VPC), which is also associated with time perception and the sense of self (Cabeza, Ciaramelli, & Moscovitch, 2012). Emotion can promote long-term episodic memory by increasing attention (Phelps, 2004) and vivid episodic memories can be recalled after a long time when strong emotion was involved during encoding (Talmi, 2013).

2.2.3 Retrieval

Episodic memory retrieval relies on conscious recollection (Tulving, 1983). Recollection is a process that elicits the retrieval of contextual information about a particular event or experience that has occurred, and it depends on how well an episode is encoded and stored. Also, context or item cues that associated with the event can trigger remembering an episode, e.g. a similar associated semantic knowledge, emotion, auditory, olfactory and visual factors. The first stage of retrieval involves a rapid and unconscious interaction between the cues and HPC, which in turn reactivates the related neocortical representations. And the second stage, a slow process, involves the manipulation of cortical networks from the output of the first stage that reinstate

previously encoded episodic memories (Moscovitch, 2008; Moscovitch et al., 2016). The retrieval of contextual and temporal information requires especially the right hemisphere of the PFC (Preston & Eichenbaum, 2013), which is also necessary for other higher order functions, for instance, organization, and executive functions. Besides, superior and inferior parietal regions engage in episodic retrieval as well to determine the cues and the recollection process, respectively (Cabeza, Ciaramelli, Olson, & Moscovitch, 2008; Yazar, Bergström, & Simons, 2012). When it refers to remembering very vivid autobiographical memories, even after many years, HPC remains always activated (Bonnici et al., 2012).

2.2.2 Consolidation

Before memories become stored for long-term, they undergo a procedure of consolidation, by which, the encoded episodic traces get stabilized and strengthened. HPC is crucial for episodic memory formation, but after consolidation, some memories become independent of this structure, and eventually seem transferred to permanent storage. Patients with hippocampal defects still maintain the ability to recall past event memories from a long time before, but they have problems to keep newly encoded episodes for long-term storage (for example the famous amnesia patient H.M.). Since those patients still have remained a relatively intact neocortex, it was thus suspected that long-term episodic memories are eventually stored in our neocortex and that this storage requires a support or transfer of information from HPC. Indeed, recent evidence from neurobiology confirmed that long-term memory is stored in dendritic spines within the neocortex (Hofer, Mrsic-Flogel, Bonhoeffer, & Hubener, 2009).

Regarding episodic memory consolidation, the trace transformation theory, an extension of multiple trace theory, posited that episodic features were quickly and sparsely encoded in HPC, where the ensembles of hippocampal neurons act as an index of neocortex representations. With the reactivation of hippocampal-neocortical ensembles, episodic memories interact and integrate into cortical semantic representations (schema), while some gist memories are posited to coexist with hippocampal representations that remain the related contexts (Y. Dudai, 2012; Inostroza & Born, 2013; Winocur, Moscovitch, & Bontempi, 2010). This does also explain

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why functional neuroimaging studies observe HPC activation in healthy subjects recalling remote autographical memories (Bonnici et al., 2012; Viard et al., 2010).

Considering the active system consolidation theory of sleep, which presumes that memory transformation between HPC and neocortex happens during sleep, gives thus sleep a fundamental role in the consolidation of hippocampus-dependent episodic memory. Rodent studies have confirmed that after sleep rats showed exploration pattern that may indicate episodic-like memory (Inostroza et al., 2013; Oyanedel et al., 2014). Importantly, the Study I included in this thesis provides the first evidence that sleep contributes directly to "What-Where-When" episodic memory in humans, which we replicated in the Study II in both children and adults.

Experimental paradigms to assess episodic memory

According to the original concept, episodic memory refers to the mental recollection of a specific event that happened at a specific time and place in the past (Tulving, 1972). Therefore, the experimental paradigm that tests the binding of the three central elements - "What", "Where" and "When" - has been wildly utilized to assess episodic memory (WWWhen paradigm, to distinguish from WWWhich, see below). Operationalized approaches to study episodic memory that are less biased to human concepts are used in animal research, and crucially, those approaches can establish equivalent indications of memory in animals that are analogous to human aspects of episodic memory. For example, episodic-like memory in rodents can be analyzed by the time that an animal spends on exploring objects in a specific environment, e.g. a maze or an open arena. The differences in exploration time on novel or familiar objects allows us to judge if an animal can recognize the object, or can distinguish where the object was located (dislocated *vs.* stationary), and even if it was seen at either an old or recent episode (Fellini & Morellini, 2013; S. M. Holland & Smulders, 2011; Kart-Teke, De Souza Silva, Huston, & Dere, 2006; Zhou & Crystal, 2009). Although the temporal component is a central feature of episodic memory, it is still under debate about how to assess this especially this component since many concepts of temporal memory are also able to explain the observed

behavior (Martin-Ordas & Call, 2013). The paradigm mentioned above considers the "when" indicates "in which moment" in time, which represents the temporal context that an event happens. There are other studies arguing that episodic memory must include a flexible mental-time-traveling, which requires subjects to remembers "how long ago" an event has happened (N. Clayton, 1998; N. S. Clayton, Yu, & Dickinson, 2003; Roberts et al., 2008), or the temporal sequence of the events in relation to each other (Ergorul & Eichenbaum, 2004; Fortin, Agster, & Eichenbaum, 2002).

There is also a debate about whether this temporal component is actually needed to form episodic memory. Eacott and Easton invented the "What-Where-Which" paradigm (WWWhich) (Eacott, Easton, & Zinkivskay, 2005; Eacott & Norman, 2004). They argued that it is rather the contextual cue that defines a specific occasion and to a lesser degree the relational or temporal (i.e. how long ago) information being a weak cue for episodic memory (Easton, Webster, & Eacott, 2012). However, Cheke and Clayton argued that even though the recollection of "when" an event occurred may not be required to recall episodic-like memory, this component is necessary to behaviorally confirm an event is a specific episode, rather than timeless facts about an object or its space (L. G. Cheke & Clayton, 2010).

The item-source paradigm is a simplified version of the above WWWhen or WWWhich. It treats the spatiotemporal context or other contextual information as a source to differentiate one event from the others without distinguishing the context into further categories (like spatial or temporal). As an everyday example, one might see a familiar face on the street, but could not remember who s/he is, and then you started searching this face in all your stored sources in mind. When in the end one finally finds the source that matches this person, you remember who s/he is and where/when you have met before. This cued recollection procedure refers to two processes. Firstly, you recognize the item (e.g., the face/person) is familiar, and then you remember the source (where/when) that you have seen this item.

The Remember/Know paradigm that is testing recognition memory has been used in the early explorations of episodic memory. Similar to the item-source paradigm, participants are asked about their personal experience on given items, and they need to report whether they

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remember that this object that they have seen before or they just *know* it (i.e. not remembering the source of the information). Remembering reflects a conscious recollection of the experienced events, and therefore is considered to reflect episodic memory. But simply knowing the object is familiar, without recollecting the episodic details would not be considered to reflect episodic memory, but only familiarity instead (Yonelinas, 2001)

Different experimental paradigms may result in different results. A study had compared different kinds of paradigms (What-Where-When, Source Memory, and Free Recall) to assess episodic memory (L. Cheke & Clayton, 2013). The inconsistent results between various paradigms revealed from this study suggested that completing different episodic tasks might not refer completely to the same cognitive functions. This evidence thus cautions the comparison among distinct studies to explain every aspect of episodic recollection, and it also suggests to rather using several tasks that cover episodic memory under different aspects.

The works in this thesis also tested episodic memory by using operationalized paradigms, which closely match the animal experimental paradigms but apply them to humans. Furthermore, by using different designs to test various aspects of human memory experience allows us to test the relation of those aspects to each other. Thus this further bridges the gap between the human and the animal experience and capabilities in episodic memory.

Developmental trajectories of episodic memory

Very early in our lives, we experience abundant episodes that relate to our daily life are even vital for surviving. For example, it is crucial for an infant to recognizing the mother's voice (Decasper & Fifer, 1980) and face (Bushnell, Sai, & Mullin, 1989). The ontogeny of hippocampus-dependent memory was suggested to start around the age of 9 months (Mullally & Maguire, 2014), when infants at this age can recollect actions over a long-term period (Carver & Bauer, 2001), with brain activities related to this recollection process (Paller & Kutas, 1992). However, as adults, we can barely remember any of those valuable episodes from infancy, which has been termed as *infantile amnesia* (Howe & Courage, 1993). To successfully form episodic memory, a broad range of cognitive functions are needed to be developed,

including relational binding, a subjective sense of self in time, and a developed spatial cognition. Therefore, it is assumed that not until the related brain structures are well developed to support the above functions, it is the developmental time point that episodic memory could be successfully stored into a long-term memory system (Mullally & Maguire, 2014).

In Tulving's original definition, autonoetic consciousness, i.e., consciously aware that an event is "remembered" but not just simply "known", is required for episodic memory (Tulving, 1972). The requirement of autonoetic consciousness thus also relies on semantic knowledge and thus makes semantic memory also an essential aspect to be assessed in parallel with episodic memory, also given the fact that semantic memory can mask a true assessment of real episodic memory (Tulving, 2005). Agreeing with this concept and accounting for semantic knowledge, some studies claimed that a true episodic memory capability only emerges around the 4th year of age and onwards (Perner, 2001; Perner & Ruffman, 1995). Even though language can help us access memory, does it mean that children do not have episodic memories before they master a language well enough to express their memories? Studies on even younger children spoke against this notion. They showed that by a benefiting from an already developed sense of self being attributed to the fact that they have autobiographical memories, children as young as two-year-old must have the capability to form episodic memory, even if their capacity to maintain this kind of memory is rather poor (Howe & Courage, 1993, 1997; Scarf, Gross, Colombo, & Hayne, 2013). Nevertheless, comparing with adults or older children (e.g., 7-year-olds), the relatively poor performance of young children (e.g., 4-year-olds) could due to their limited capacity to encode context information and complex relational structures (Yim, Dennis, & Sloutsky, 2013). The capacity to retrieve specific episodes continually improves during middle childhood, while memory for isolated items or facts reaches the adult level earlier than memory for contexts (Ghetti & Angelini, 2008; Picard, Cousin, Guillery-Girard, Eustache, & Piolino, 2012). This developmental change of episodic memory emerges from the parallel development of a brain network that includes the HPC, PFC and posterior parietal cortex (Ghetti & Bunge, 2012). Inspiringly, this early period of episodic memory development in the middle of childhood and beyond is the time where environmental support that is provided

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by educational settings may have particularly large and beneficial effects on memory performance (Sander, Werkle-Bergner, Gerjets, Shing, & Lindenberger, 2012).

The major consolidation mechanism underlying the development of episodic memory in children is unknown. Does sleep support episodic memory the same way as it is in adults? And if so, would the effects of sleep on episodic memory be comparable as it is in declarative memory in the similar age group children (e.g. Wilhelm, Prehn-Kristensen, & Born, 2012)? Hence, in the Study II of this thesis, we addressed those questions not only in adults but also in children of 8-12 years as a first step of episodic memory development at the ages towards a fully developed adult episodic memory.

Interdependency of semantic memory and episodic memory

As another typical declarative memory, semantic memory cannot be ignored when understanding episodic memory. Semantic memory is defined as being independent of any form of a specific memory experience. It refers to general knowledge about the world, storage of "words and other verbal symbols, their meaning and referents, about relations among them, and about rules, formulas, and algorithms for manipulating them" (Tulving, 1972; Yee, Chrysikou, & Thompson-Schill, 2013). Examples of semantic memory include names and attributes of all objects and actions, concepts and the association between them, categories and their bases, knowledge of causes and effects, and so on (Binder & Desai, 2011). Semantic memory is thus important for the formation to episodic memory since we cannot remember the past, plan the future or reason about information without having a preexisting conceptual knowledge stored in the semantic memory system (Binder & Desai, 2011).

It has been long assumed that to process semantic concepts partly depend on the sensory and motor experiences. For example, when we process motion, sound, olfaction and gustatory concepts, the related brain areas (or nearby) also active as during the real sensory or motion actives (See a summary in Binder & Desai, 2011). On top of those direct sensory-motor related concepts, those abstract representations were suggested to be supported by the "convergence

zones" that refer to inferior parietal cortex, ventral and lateral temporal lobe and anterior portions of fusiform gyrus (Binder & Desai, 2011; Damasio, 1989)

Although Endel Tulving differentiated episodic memory and semantic memory at the very early stage, with the formation of semantic memory not necessarily depended on episodic memory (Tulving, 1972), there is still an ongoing debate regarding to what extend semantic memory is formed from episodic memory (Yee et al., 2013). Studies in children with amnesia at an early age of life revealed evidence that, although those children had episodic memory impairments that due to bilateral hippocampal damage, their semantic knowledge was relatively intact (Bindschaedler, Peter-Favre, Maeder, Hirsbrunner, & Clarke, 2011; Gardiner, Brandt, Baddeley, Vargha-Khadem, & Mishkin, 2008; Vargha-Khadem et al., 1997). Moreover, evidence that supports the interdependency of these two kinds of declarative memories is accumulating (see the review from Greenberg & Verfaellie, 2010).

It was generally accepted that the neural basis of semantic memory and episodic memory are different, while episodic memory depends primarily on the hippocampus, semantic memory largely depends on the underlying cortices (Vargha-Khadem et al., 1997). However, these two memory types are not completely independent, rather, they support the formation of each other. At encoding, semantic memory can act as a framework or scaffolding to facilitate the episodic acquisition, which has been found in healthy subjects compared to patients with an impaired semantic system like dyslexia and aphasia (Graham, Simons, Pratt, Patterson, & Hodges, 2000; Kan, Alexander, & Verfaellie, 2009; Kinsbourne, Rufo, Gamzu, Palmer, & Berliner, 1991; Reder, Park, & Kieffaber, 2009; Y. Wang, Mao, Li, Lu, & Guo, 2016; Ween, Verfaellie, & Alexander, 1996). Other works suggested that new semantic learning could also rely on the function of the MTL thought to be the core structure supporting episodic memory. For example, amnesia patients had impaired abilities to learn new semantic knowledge, which also correlated with the degree of MTL damage (Levy, Bayley, & Squire, 2004). In fact, one theory suggests that semantic knowledge is abstracted by multiple various spatiotemporal contexts (Baddeley, 1988; Greenberg & Verfaellie, 2010; Mayes & Roberts, 2001). Even though it seems possible to form semantic memory independent of MTL or the capacity for episodic encoding (Stark,

Gordon, & Stark, 2008; Tulving, Hayman, & Macdonald, 1991), such a formation is rather slow and the learned new information is then often hyper-specific and cannot well integrate into the semantic storage (Greenberg & Verfaellie, 2010).

This mutual interdependent effect has also been found in the retrieval phase. Again, studies that compare healthy subjects with amnesic patients have shown that the MTL impairment in patients had harmed their autobiographic memory and therefore hindered their ability to access semantic knowledge (Kopelman, Stanhope, & Kingsley, 1999; Westmacott, Black, Freedman, & Moscovitch, 2004). Likewise, autobiographical memories also construct semantic knowledge that when the capacity of episodic recall declines, semantic memory also declines (Maguire, Kumaran, Hassabis, & Kopelman, 2010; Piolino et al., 2003). Additionally, evidence from neuroimaging studies showed co-activation in mutually shared brain regions when processing these two kinds of memory. For example, the posterior cingulate gyrus and adjacent precuneus may function as an interface between semantic networks and the HPC to facilitate meaningful events into episodic memory (Binder & Desai, 2011). Indeed, recent evidence from (Brodt et al., 2016) suggested that with each repetitive encounter of episodes within a virtual spatial maze, the activation of HPC network decreases while posterior parietal cortex increases. It thus might mark a quick transition from the hippocampal dependency of episodic memory to a stable neocortical semantic network during learning. Together with the additional finding that this heightened parietal cortex activation was predictive of the memory performance on long-term (i.e. a day), it opens the possibility that such a fast memory transfer could also happen during wakefulness when similar episodes of the day are spontaneously activated, a process thought to happen more often during sleep.

In summary, scientists reached a consensus that episodic memory and semantic memory are not entirely distinct mental process. On the contrary, they facilitate each other's formation and recall. Since these two memories also have different features and function, the mechanism regarding how they mutually support each other is still an interesting and important scientific question. The Study III in this thesis slightly touched this topic by designing a task with semantically related word pairs with episodic temporal context. It was not aimed to disentangle

the relationship between semantic memory and episodic memory in specific but could provide some interesting hints that refer to age-related differences in memory consolidation that are due to the diverse of memory types.

3. Sleep

We are all born with a natural ability to switch our states between wake and sleep. During wakefulness, we receive information, form memories, and express emotions with consciousness. On the contrary, when falling into the state of sleep, we lose consciousness and the awareness of what happens to our body during sleep. Having to give up the apparent loss of wakeful benefits for about one-third of our lifetime marks the importance of sleep in our survival. Although it has been for long taken as granted, sleep is crucial for our body health in energy saving (Berger & Phillips, 1995), metabolic regulation (Knutson, Spiegel, Penev, & Van Cauter, 2007) and adaptive immune functions (Lange, Dimitrov, & Born, 2010). More importantly, sleep speaks strongly for the notion of being mainly "for the brain" (Hobson, 2005).

Sleep in mammals consists of two main stages: NonREM sleep (including Stage II sleep and SWS) and REM (rapid-eye-movement) sleep, which alternate in a cyclic manner. NonREM sleep is dominant for the early part of a typical night sleep, and its proportion decreases while REM sleep becomes prevalent at the second half night until waking up.

Sleep patterns change dramatically across the lifespan. For humans, the amount of sleep decreases gradually with age, from about 16 hours at neonate to about 6 hours for the elderly after 70-year-olds. However, the most dramatic change happens before adolescence (Roffwarg, Muzio, & Dement, 1966). During the early postnatal period, sleep is immature, and is separated in quiet sleep (analogous to adults' NonREM sleep) and active sleep (analogous to adults' REM sleep). With age, REM sleep gradually diminishes to about 25% by the age of 2-3 years, when NonREM sleep becomes dominant. During childhood, sleep duration gradually drops to about nine hours at around 12 years of age (Iglowstein, Jenni, Molinari, & Largo, 2003).

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Sleep and memory consolidation

It has been widely accepted that sleep is important for memory consolidation (Diekelmann & Born, 2010; Rasch & Born, 2013). A vast body of evidence showed that compared to wakefulness, sleep positively supports declarative memory consolidation (e.g., Plihal & Born, 1997), including the formation of semantic memory (e.g. Tamminen, Lambon Ralph, & Lewis, 2013; Tilley, Home, & Allison, 1985), and also non-declarative memories like motor learning (Antony, Gobel, O'Hare, Reber, & Paller, 2012; Brawn, Fenn, Nusbaum, & Margoliash, 2008; Fischer, Hallschmid, Elsner, & Born, 2002) and emotional memory (P. Holland & Lewis, 2007; Hu, Stylos-Allan, & Walker, 2006). New findings suggested that sleep specifically benefits the episodic kind of memory (Inostroza et al., 2013; Inostroza & Born, 2013; Oyanedel et al., 2014).

Starting with pioneer works of Ebbinghaus (Ebbinghaus, 1983), the forgetting rate of learned syllables was reduced after a night of sleep, and follow-up studies confirmed that sleep prevents further forgetting (Jenkins & Dallenbach, 1924). Therefore sleep has been assumed to act as passively protecting memory from retroactive interference (J. M. Ellenbogen, Payne, & Stickgold, 2006). This hypothesis reinforced that sleep transiently shelters memory from interference, but it denied that sleep actually consolidates memory. However, if memory remains unchanged during sleep, it would not show a reduced susceptible to interference after sleep (Jeffrey M. Ellenbogen, Hulbert, Stickgold, Dinges, & Thompson-Schill, 2006). Hence sleep must play more than a simple role of "passive protection".

Recent theories suggest an active role of sleep in memory consolidation (see the review of Diekelmann, 2014; Diekelmann & Born, 2010; Rasch & Born, 2013). According to the active system consolidation theory, during learning, information is encoded in parallel to HPC, as the temporary memory hub, and the stimuli-relevant neocortex, as the long-term storage of memories. During sleep, newly encoded hippocampal memory representations become reactivated and reorganized, which allows newly acquired information to be gradually integrated into the pre-existed neocortical neural network for long-term memory system (Rasch

& Born, 2013). This hypothesis has been updated recently for episodic memory consolidation. It assumes a leading role for the prefrontal-hippocampal memory system that during wakefulness hippocampus encodes the episodic natures by binding an experienced event into its temporal-spatial context (Huber & Born, 2014). During subsequent sleep (presumably SWS), neural memory representations in the hippocampus (e.g., place-cells) become repeatedly and spontaneously reactivated in the same temporal order as during the experience (i.e., so-called memory "replay"), allowing for their redistribution largely into neocortical and striatal networks (Huber & Born, 2014; Inostroza & Born, 2013). This process could also result in a strengthening of the overlapping gist across memories to form our cognitive schema, but at the expense of weakening associated additional information, for example, the learning context (Lewis & Durrant, 2011). Therefore sleep has been considered to function for "semantization" with episodic details being pruned and reorganized to form more abstract semantic knowledge. Although there is evidence that one-night sleep could not be enough to reveal such a "semantization" effect (Cox, Tijdens, Meeter, Sweegers, & Talamini, 2014; Jurewicz, Cordi, Staudigl, & Rasch, 2016).

Sleep stages and specific features in memory consolidation

Substantial evidence supported the dual process hypothesis that hippocampus-dependent declarative memories preferentially benefit from SWS, whereas non-declarative memories, such as procedural memory and emotional memory, additionally profit from REM sleep (Born, Rasch, & Gais, 2006; Gais & Born, 2004). Since this thesis mainly focuses on episodic memory, a main kind of declarative memory, some important and relevant sleep features are discussed below.

According to the active memory consolidation model, newly encoded memory representations that temporarily are stored in hippocampal networks, are reactivated during SWS after the encoding experience to be redistributed and transferred to cortical networks severing as a long-term store. This hippocampal-neocortical transfer of reactivated memories is thought to be orchestrated by EEG slow oscillations (Mitra et al., 2016) that are generated in the

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neocortex and stimulate synchronous hippocampal reactivations that are marked by hippocampal sharp-wave ripples (SW-Rs), and thalamo-cortical sleep spindles (Marshall, Helgadottir, Molle, & Born, 2006). Hippocampal ripples nested in spindle oscillations (Clemens et al., 2007; Staresina et al., 2015), i.e., spindle-ripple events have been proposed as a mechanism that specifically serves the transfer of reactivated hippocampal memories to neocortical, preferentially prefrontal cortical areas (Born & Wilhelm, 2012).

Key features of sleep architecture that underlie memory consolidation thus include slow oscillations (SOs, also called slow waves) (Chauvette, Seigneur, & Timofeev, 2012) and sleep spindles (Sirota, Csicsvari, Buhl, & Buzsaki, 2003), which also develop with age. Slow-wave activity (SWA), which is defined by the 0.5- to 4.0 Hz frequency band including the core frequency of slow oscillations (SOs, ~0.8 Hz in humans), has been proposed to reflect the dynamics of synaptic strength (Tononi & Cirelli, 2006), which is associated with the degree of neural synchronization in the cortical networks (Whitlock, Heynen, Shuler, & Bear, 2006). There is a gradual increase of SWA during childhood. This is in line with the overwhelming increase in synaptic connectivity, starting from the first year of life and reaching a plateau around the beginning of puberty (McAllister, 2000).

Sleep spindles are waxing and waning activities between 12–15 Hz that are typical oscillations of light sleep (Stage 2 sleep) as well as deeper SWS (De Gennaro & Ferrara, 2003). Spindle activity can be detected within the first month of life (Ellingson, 1982). Spindle densities (highest at centroparietal sites) slightly decrease towards the end of infancy (2-3 years), and then increase strikingly during the whole childhood until puberty, to then slowly decrease again with age (Scholle, Zwacka, & Scholle, 2007) though between the ages from 2-5 years in a longitudinal study no such change was observed (McClain et al., 2016).

SWA and sleep spindles that associate with hippocampal SW-Rs are important sleep features, and their occurrence and properties can reflect local synaptic networks that were previously strengthened (e.g. LTP) during a wakeful learning experience (Huber, Ghilardi, Massimini, & Tononi, 2004; Werk, Harbour, & Chapman, 2005). There is also further indication that the coordinated occurrence of such sleep features thought to support the

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hippocampal-to-neocortical transfer of memory representations is a causal predictor for declarative memory consolidation (H. V. Ngo, Claussen, Born, & Molle, 2013; H. V. V. Ngo et al., 2015). The developmental trajectory of these sleep features could serve as a reference for the development of memory consolidation mechanism and for the developmental state of the memory system itself.

Developmental evidence on sleep and memory consolidation

Childhood is the most important period of our life to learn and to shape the connections in our brain for all future experiences. Children spend much of their waking hours to acquire skills and knowledge of the world. This requires a superior capacity to learn and form memories that benefit naturally from the developing brain, which is assumed to be very sensitive to novel stimuli and experiences (Wilhelm et al., 2014). Remarkably, childhood is also the period when the most dramatic changes of sleep structure happen (Roffwarg et al., 1966). Children spend much longer time in sleep and sleep deeper than adults. Considering of the memory function of sleep, it is reasonable to assume that sleep plays an important role for the developing brain to build up matured cognitive functions.

Sleep for memory consolidation in children was first reported in declarative memory, where age-appropriate word-pair learning was used for 9-12 years old children (Backhaus, Hoeckesfeld, Born, Hohagen, & Junghanns, 2008). This study showed clear evidence that declarative memory in children was enhanced after sleep, but not after an equivalent length of wakefulness. Importantly, this study reported a positive correlation of the retained word pairs with NonREM sleep. Follow-up studies compared children at this age with healthy adults and indicated that the effect size of sleep on declarative memory consolidation (on word-pair and visuospatial memory) in children was equivalent to adults, even though children had much higher amounts of SWS than adults (Wilhelm, Diekelmann, & Born, 2008). Unlike the beneficial effect of sleep that was found in declarative memory, post-sleep procedural memory deteriorated in children, which was opposite to adults (Wilhelm et al., 2008). Of note, the degree of the post-sleep impairment in procedure memory failed to predict long-term

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performance (Zinke, Wilhelm, Bayramoglu, Klein, & Born, 2016), a phenomenon that was found in sensorimotor learning in young songbirds (Deregnaucourt, Mitra, Feher, Pytte, & Tchernichovski, 2005). These studies spoke against the hypothesis that more SWS or longer sleep time predicts larger sleep effect on memory. Then why do children show so much of SWS as compared to adults? A study that compared children at that age (i.e. pre-puberty) with adults shed new light on this: it was not the absolute amount of declarative memory, but rather the abstract gain in knowledge from the implicitly learned material that in particular benefited from the SWS-rich sleep in children (Wilhelm et al., 2013). Similarly, sleep also facilitates newly acquired novel words to integrate into children's long-term lexical memory and was associated with the ability to recognize and recall novel spoken words (Henderson, Weighall, Brown, & Gaskell, 2012).

Nevertheless, there are only a handful of studies that investigated sleep's effect on memory in younger children and infants for declarative memories. Those studies mainly utilized a task paradigm called "differed imitation" – an experimental paradigm to test declarative-like memory in infants for unique experiences. For this paradigm, a short nap shows benefits for declarative memory consolidation (Seehagen, Konrad, Herbert, & Schneider, 2015), and the flexible memory retrieval in infants (Carolin Konrad, Seehagen, Schneider, & Herbert, 2016), as well as differences along the early age for the role of night sleep in crucial aspects of the recall for the imitation (C. Konrad, Herbert, Schneider, & Seehagen, 2016). Also, sleep seems to facilitate the generalization/abstraction capacities in infants (Friedrich, Wilhelm, Born, & Friederici, 2015; Gomez, Bootzin, & Nadel, 2006; Hupbach, Gomez, Bootzin, & Nadel, 2009).

Although studies in sleep and memory consolidation in children are accumulating, there is to date no evidence about sleep's role in particularly episodic memory consolidation in children, i.e. at an age where they show a developmental peak in SWS, which is thought to have the optimal conditions for hippocampal-neocortical memory consolidation processes taking place. This makes pre-puberty childhood an optimal model to study hippocampus-dependent episodic memory consolidation and is thus the focus of investigation in this thesis.

Objectives and expected output of the doctoral research

To sum up, there was a knowledge gap about sleep's role in the consolidation of episodic memory, e.g., how does sleep support episodic memory and its different aspects (e.g., temporal or spatial context), and how does episodic memory develop from childhood to adulthood. This doctoral thesis aims to explore how episodic memory consolidates during periods with either nocturnal sleep or daytime wakefulness, and how this process is related to the aspects of childhood brain development. For this purpose, we planned the following experiments:

First of all, a novel experimental paradigm was established to test episodic memory in the strictest definition ("What" happens at "Where" and "When"). This task allowed us to examine episodic memory in children and adults with two behavioral measures that is an explicit measure by oral report, and an implicit measure by tracking eye movements. Both measures allowed the assessment of the spatiotemporal integration that was required to test specifically for episodic memory in a verbal and non-verbal manner as well as to track the interdependency of these two measures and how each depends on consolidation processes. In addition, the combination of both explicit with implicit measures bared the potential for an arbitrary use of both measures to indicate core features of episodic memory in future applications using solely eye-tracking to measure episodic memory, e.g. in patients with language deficiencies or infants. The experimental paradigm is described thoroughly in Study I (Appendix - Study I). In this study, the effect of sleep on memory recall and the possible neural basis during sleep that could contribute to the memory consolidation process were disentangled. According to the assumption that overnight sleep facilitates declarative memories (Diekelmann & Born, 2010), we hypothesized that after sleep, participants would recall more episodic memory for the explicit measure. Also, an animal study has reported that after sleep rats showed stronger behavior indicating episodic-like memory (Inostroza et al., 2013), so we explored the possible link to that study to measure spatiotemporal integration of episodic memory in humans using eye-tracking of exploration behavior similar to the task design used in rats. We attempted to

establish an implicit measure that is sensitive enough to detect episodic memory for non-verbal subjects, as good as the oral report.

Next, we tested the hypothesis that episodic memory consolidation happens preferably during SWS. Since SWS is naturally more abundant in children, and no study has shown how sleep supports episodic memory particularly in children, we adopted the experiments from the first study ("What-Where-When" paradigm) and utilized it also to children aged 8-12 years. In addition, to amend the shortage of the previous study design that lacked a control on shorter forgetting periods, additional groups of children and adults were recruited to serve as the pre-sleep control. This additional testing before sleep on a shorter time interval after encoding attempted to understand the dynamics of forgetting or change of episodic memory between short-term and long-term intervals. The results are reported in Study II (Appendix - Study II). This study provides an overview of how episodic memory changes from the onset of sleep towards longer time intervals after nocturnal sleep or daytime wakefulness. Also, a direct comparison between memory performance of children and adults was presented. We hypothesized that there is a significant sleep effect for the What-Where-When episodic binding in children as well, with the strength of the sleep effect being positively influenced by the amount of SWS or SWS related electrophysiological features, such as SWA or SO densities as well as sleep spindles. Furthermore, when compared with the adult subjects on recall performance after long and short time intervals, the sleep children were expected to outperform the sleep adults for the explicit What-Where-When episodic binding on the long interval, but only after sleep, not wakefulness, and showing less forgetting over sleep than adults.

Lastly, to exclude the possibility that the missing sleep effect on the temporal aspects of episodic memory that was masked by the strong sleep effect on the spatial aspects of the "What-Where-When" task, we adapted a classic paired-associative learning task (studying word pairs) to also allow recall for episodic aspects of the memory (temporal context, and its binding of word-pair memory as items). The word-pair learning served not only as a replication control with a previously reported benefit from sleep in children (Backhaus et al., 2008) and adults (Plihal & Born, 1997), but also diverted participants' from noticing the episodic temporal

context. Using this paradigm imitated the everyday situation in which we can still recall when an event happened even though we did not pay specific attention to remember the time when that event happened. We compared the sleep effect on these two kinds of memory in children. Additional adult groups for the long-term memory controls were also tested either before or after sleep. The procedural design was analogous to the "What-Where-When" episodic paradigm in Study II, and allowed us to simultaneously compare sleep's influence on different experimental paradigms: purely episodic item-temporal context memory, and more semantic-based memory for word-pair associate learning. The results are reported in Study III (Appendix - Study III). We hypothesized that the word-pair memory shows signs of forgetting after one hour, but with sleep further preventing this forgetting. For the episodic aspects, we expected to see children that slept recall the temporal contexts and their bound items better than the Wake children and Pre-sleep children. Additionally, we hypothesized the forgetting curve of children and adults could be similar as we observed in Study II.

Conclusion and general discussion

Taken together, the findings included in this thesis indicated that i) a nocturnal sleep benefits the binding of an item into its spatiotemporal context, a core feature of episodic memory, for both explicit and correlating implicit measures in human adults. In particular, fast sleep spindles may underline the neural basis of this consolidation (Study I). Introducing the same paradigm to children around ten-years-old, we found that ii) children did not exceed adults as we expected for the capacity of consolidating the absolute amount of episodic memory, even though they had a superior amount of SWS. This result is in line with a previous study that showed comparable sleep effect on a visuospatial memory task in children and adults (Wilhelm et al., 2008). However, by assessing the memory performance shortly after encoding, we found that in order to understand sleep's efficiency in memory consolidation, the pre-sleep performance should be taken into account (Study II). Finally, we asked whether the sleep effect on a general memory consolidation profits from the episodic nature of memory. To answer this question, we examined the consolidation of word-pair memory, which has been shown in previous studies to have robust benefit from sleep. We could replicate the previous findings of sleep's positive effect on word-pair learning in school children (Backhaus et al., 2008; Wilhelm et al., 2008). In addition, we provided new evidence that the episodic temporal context from a learning material also benefits from sleep and may be an integral part of the learning memory that aids this sleep benefit. Surprisingly, in this task, sleep did not preferentially support a fully integrated by binding its items (word-pairs) to their source (temporal context), rather, sleep seems to encourage the process of "unbinding", which could serve as additional evidence for schema building. Also, SWS and sleep spindles were found to associate with those consolidation processes differentially.

Forgetting curves differences over sleep and comparing related memory types between children and adults

When looking at Study II and Study III together, an interesting age-related difference of forgetting curves between "what-where-when" episodic memory and semantic based word-paired memory appeared. In Study II, the adults forgot episodic memory over all measured time points, even after one night of sleep. However, in children the recall of episodic memory kept rather steady, starting with lower performance shortly after encoding (~1 h), but on the long time interval (~10 h) reaching recall levels comparable to adults. Notably, sleep in children upheld episodic memory to a larger extent, though long-term forgetting did not reach significance (Figure 1, Study II). On the contrary, for the word-pair memory, the memory more based on semantic knowledge, children's forgetting was more drastic over the long retention time (Figure 1B, Study III). Why did children seem to forget semantic associations over time (even after sleep), but maintain episodic associations, while this is the opposite for this two memory types in adults? To our knowledge, this is the first evidence of two opposite age-related forgetting trends for two different declarative memory types (i.e., episodic-like or semantic-like memory). Our understanding from forgetting curves stems mainly from the pioneering work from Ebbinghaus (Ebbinghaus, 1983). He and later researchers following his work were testing repeatedly encoded nonsense syllables in adults with different retention time. They have observed a consistent and dramatic forgetting over time (Murre & Dros, 2015). Importantly, on top of the general forgetting, a slight improvement of memory appears around 24 h or shortly after, an effect, which has later been attributed to the sleep (Jenkins & Dallenbach, 1924).

Our adults' performance on episodic "What-Where-When" memory showed a similar pattern, which precludes the influence of familiarity of the learning materials (familiar face *vs.* less familiar nonsense syllables) and mental efforts (implicitly encoded episodes vs. repeatedly learned syllables). However, should children follow a similar forgetting curve regardless of the memory type? To our best knowledge, there is no previous evidence that has described how

memory in children changes within 24 hours. However studies on eyewitness memory tracked over weeks to month indicated that children and adults share similar accuracy (incorrect-to-correct rate) for the long-term memory (Paz-Alonso, Larson, Castelli, Alley, & Goodman, 2009; Pipe & Salmon, 2009). This seems to back up the episodic performance for our Sleep and Wake groups, which did not show significant differences between age groups. However, can the most significant difference between age groups after the short 1-h interval be explained by the encoding efficiency, i.e., children encode worse than adults, or show a fast forgetting, i.e., children could lose those memories more quickly within the one hour? Given that the encoding in children was generally not impaired as compared to adults (Word-pair memory immediate recall, Figure 2A) and episodic memory is a rather volatile memory, the weaker episodic component of Study III seems to have deteriorated already after 1-h, and then remained similar level after 11h (Word-pair and list memory, in Figure 2C). This could be considered evidence for the fast episodic forgetting of specifically episodic memory in children over short periods. From this we could infer that the huge difference observed in the WWW memory in the pre-sleep groups between the children and the adults could due to the fast forgetting of children, whereas the lessened forgetting of the word-pair memory (Study III, Figure 1B, 2B) could due to the different encoding strategies owed to the different nature of those two memory types (more semantic related, less episodic related). Nevertheless, we cannot rule out that children encoded less contextual episodic details in the first place, and thus future studies that thoroughly design to test this assumption should be conducted.

Limited capacity of sleep on memory consolidation

When focusing on the quantitative change of memories other than the exact memory type, we found there are interesting consistencies between Study II and III. For instance, in Study II, the group that performed higher before sleep (i.e., the Pre-sleep adults reached over 60% performance) had reduced memory after sleep, while the low starters (i.e., the Pre-sleep children reached less than 30%) did not significantly differ (and even slightly increased) after sleep; In Study III, learners with no forgetting before sleep (Pre-sleep children) dropped

significantly as compared to after sleep, while the Pre-sleep adults who already forgot before but slightly upheld the memory after sleep. In other words, independent of memory type and subjects' age, the pre-sleep performance may predict the sleep effect on memory consolidation. Thus, the higher performers lost over sleep, while the more moderate performers did not. This is in line with previous studies that claimed sleep favors specifically the intermediate performance before sleep (Wilhelm, Metzkow-Meszaros, Knapp, & Born, 2012). In this study, children and adults were trained for motor sequence learning. Comparing with wakefulness, sleep effect was only significant for the intermediate pre-sleep performers, rather than the high and low performers. Also, a recent study in adults on the sleep's effect of information load during encoding adds new evidence on declarative memory to support this idea (Feld, Weis, & Born, 2016). In this study, the beneficial sleep effect was only observed when participants learned an intermediate amount of 160 word-pairs, but not in either shorter list (40 pairs) or longer list (320 pairs). Notably, the conclusions of these two studies all speak for a limiting effect of sleep on over-trained ceiling performance or material learned under high mental demands, as it was suggested previously that sleep's enhancement is greater for weakly encoded than strongly encoded memories (Diekelmann, Wilhelm, & Born, 2009). Our tasks required less cognitive load with either one-time encoding of word-pairs, or implicitly encoded episodes and also precluded ceiling performance. In fact, the immediate recall performance of word-pair for both children and adults was set to an intermediate level (Study III, Figure 2A), which allowed sensitivity for a sleep effect. It might thus be that particularly in children the cognitive load during encoding and the memory level at the time of going to sleep were ideal to benefit from it, and those conditions were met to a lesser degree for adults. Moreover, the fact that children retained similar amounts of episodic memory than adults after a long retention, even without sleep, supports the view that there are memory consolidation mechanisms that might protect memory at a very low level independent of processes during sleep, at least for time scales of ~10 h. Although with no direct evidence within the first 24 h, research on children for eyewitness memory had revealed that young children tend to remember less information of an event, but with a higher accuracy (Ornstein, Gordon, & Larus, 1992; Poole &

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White, 1995), which indicated children's limited capacity of remembering that information but relatively good ability to maintain it over a long-term period.

Sleep for vivid episodic remembering, episodic forgetting and the relation to semantic memory

The active systems consolidation theory predicts that the hippocampus-dependent representations are gradually integrated into the neocortex and become less hippocampus-dependent by spontaneously reactivation during sleep (mainly during SWS), which is a process that favors memory to be less vivid (i.e. less bound into the context) and fosters abstractions to form cognitive schema (i.e. unbound item memory) (Inostroza & Born, 2013; Lewis & Durrant, 2011). Does this mean that sleep sacrifices episodic memory to favor semantic memories? It has been assumed that sleep has a "trade-off" effect between different memory types (Diekelmann, 2014). For example, it has been found that after sleep, the emotional component of a scene increased, but the memory for the neutral background concurrently decreased (Payne, Chambers, & Kensinger, 2012; Payne, Stickgold, Swanberg, & Kensinger, 2008). How this preferential consolidation of sleep works in emotionally neutral hippocampus-dependent memories is not well studied yet, but from the data presented in this thesis, we may speculate about this further.

In Study I, we showed that there is a clear sleep effect for a better vivid episodic memory (not only the "What-Where-When" but also the "What-Where" memory) as compared with wakefulness, which sounds contradicting the assumption that sleep weakens episodic memory. But when considering the pre-sleep memory performance in Study II, a clear forgetting in episodic memory was demonstrated in the sleep group for adults. That is to say, sleep in adults prevents further forgetting of vivid episodic memory. However the current episodic task was not designed to measure a simultaneous increase of semantic memory, thus we still do not know whether there are direct sleep-processes involved in transferring episodic memory to semantic memory. Surprisingly, children did not show such an over-night forgetting of episodic memory. On the contrary, children that slept were even slightly better than the children measured for

pre-sleep performance. Thus, the hypothesis that sleep promotes the process of forgetting hippocampus-dependent episodic context (Hardt et al., 2013) does not seem to apply to children in this task, since their memory was still bound into context as it was before sleep. However, the evidence in Study III did speak for an episodic "unbinding" role of sleep in children, because after sleep the "episodic-like" memory (word-pair and list memory) did not change whereas the separate measures including word-pair or list memory became significant. Though this study cannot directly proof any memory transformation over sleep, the implication of the "unbinding" between memories could serve as first evidence to guide future studies on that matter.

Though future studies should design dedicated test to address this issue more accurately, we are tempted to speculate that one-night of sleep (or the early following nights after episodic encoding) benefits the remembering specifically of vivid episodic memory when comparing with the same period of wakefulness. But with the passage of time and more sleep periods, the original episodic details would become obscure and the core content (fact) of what happened of an experienced episode could become less dependent on the hippocampus and ultimately form the semantic knowledge stored mainly in cortical networks. In terms of development, this process might be speeded up in children and their sleep, as the nature of being young is to learn more and build up individually new cognitive schema.

The candidates of sleep parameters that support episodic memory consolidation

The sleep EEG data did not reveal any consistencies regarding the neural mechanism that may underlie the consolidation of explicit "What-Where-When" episodic memory, but only for an association of sleep spindles to the implicit measure. It has been found that in humans, SWS-rich sleep early during the night consistently benefits (explicit) declarative memory (Diekelmann & Born, 2010). Combining this with the evidence of neural reactivation in the hippocampus during slow-wave sleep (Wilson & McNaughton, 1994), SWS has been assumed to be important for long-term storage of episodic memory (Inostroza & Born, 2013). Unexpectedly, according to our design, the hallmark features of SWS like slow oscillations (SOs) and slow-wave activities (SWA) did not reveal any consistent correlations that could

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underpin the consolidation of episodic bindings, in neither adults nor children. This unanticipated outcome, however, does not exclude the real contribution from SWS to episodic memory consolidation.

On the one hand, to avoid reconsolidation process that could be triggered by repeatedly assessing memory (Hupbach, Gomez, & Nadel, 2009), we avoided testing the subjects repeatedly on previously learned episodes. This resulted in the lack of a baseline measure to indicate the individual change in memory over a long retention interval. Thus our episodic task design failed to offer the possibilities for an apparent association with potential EEG features. Even though at Study II, we account for this defect by adding the Pre-sleep groups to serve the baseline for behavior comparisons, the design was still not appropriate to report the exact contributor of sleep parameters for episodic memory consolidation on an individual level.

On the other hand, the experimental paradigm with baseline measure in Study III allowed proper associations of memory changes with sleep features. This showed positive associations for the episodic binding of word and list memory with SWS and spindle activities that was contrasted by a negative association of sleep spindles with the semantic content of this memory (i.e., for the word-pair memory itself). Indeed, new evidence from (Niknazar, Krishnan, Bazhenov, & Mednick, 2015) revealed the concurrence of spindle activity and slow waves during SWS might be optimally suited for hippocampus-dependent memory consolidation (Maingret, Girardeau, Todorova, Goutierre, & Zugaro, 2016; Niknazar et al., 2015). In addition, there is new evidence that, even disregarding the lacking baseline, the episodic memory recall after sleep in adults seems associated with the infra-slow organization of sleep spindles predominant in parietal cortical areas (Lecci S. et al., Manuscript in revision), i.e. within the same cortical areas that support long-term memory following rapid encoding of re-experienced episodes (Brodt et al., 2016). Given that sleep spindles seem to play a role in episodic memory consolidation as observed here, their highest density specifically during periods of childhood when episodic memory is most rapidly developing (Scholle et al., 2007) shifts the focus away from slow waves and highlight their importance for future investigations on episodic memory.

Taken together, this suggests that spindles and slow-wave sleep favor the consolidation of episodic rather than semantic memory. However the exact role of those EEG features and how they interplay to benefit one over the other aspect of memory needs to be investigated in future studies.

Limitations and outlook

Our studies provided new perspectives on the efficiency of consolidation over one night of sleep when considering the encoding level and hence revealed different forgetting curves depending on age. Importantly, by adapting different paradigm to assess episodic memory, many questions regarding sleep's role in episodic memory consolidation could be answered. But due to contradicting findings, the different paradigms also generated further questions on how the different factors determine the consolidation process of different declarative memory types in sleep. For instance, at the behavioral level, if episodic memory differed largely between children and adults already one hour after encoding, but was equivalent after 10 hours, then how do episodic memories fade on even shorter and longer time intervals (e.g. minutes and days)? Also, is the lower performance of children after one hour already an indicator of worsened encoding in this episodic task of a faster forgetting? Finally, would the episodic memory be also protected from further wakefulness (i.e. extending the retention interval to 24 h), thus revealing a true protection of the episodic memory from interference? Those questions call for future investigations to find the ideal time window for sleep to consolidate episodic memory with the highest efficiency, and how this time window depends on age.

More interestingly, what is the sleep's role in balancing between episodic memory consolidation and cognitive schema formation? It would be very interesting to discover whether sleep promotes episodic fading while in parallel forming new semantic memory/schema that are related directly to everyday episodes rather than to repetitively learned material. Though the tasks used here could indicate changes in binding of items into their spatiotemporal context on not explicitly learned materials, it could not indicate a parallel transfer of this unbinding to contribute to a schema memory.

Last but not the least, why do we forget some memories that were vividly formed and consolidated? If we forget the most details over time, what are the things (gist) that are left, and what determines the selection of the remained gist memory? And what might cause the remote vivid memories that we always remember (strongly personal emotion related)? Future studies

should address possible predictors on the fading of memory for specific items or their specific context (e.g. spatial or temporal context) and how they relate to sleep, modulating emotion, attention and the strength of the memory (e.g. influences of repetition or varying encoding time).

On the neural level, our investigation did not provide direct evidence of which sleep stages or featured sleep parameters contribute to the consolidation process of explicit episodic memory (Study II). Although SWS was hypnotized to be the key in declarative memory consolidation, it was only in the Study III that an association was found between the percentage of SWS with the word and list memory. However, there was no difference of this memory comparing between Sleep and Wake conditions. The question remains that if sleep enhances consolidation of truly episodic memories on the behavioral level, then why there is no consistent correlation across the studies reported here? We assume that the consolidation of episodic memory may refer to a comprehensive process that multiple brain regions were involved, which is hard to be detected by the sparse electrodes we applied in all these studies. In conclusion, future studies are needed to further disentangle sleep' role in episodic memory consolidation.

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Description of personal contribution

Weber, FD.*, **Wang, JY.***, Born, J. & Inostroza, M. (2014). Sleep benefits in parallel implicit and explicit measures of episodic memory. *Learning & Memory*, *21*(4): 190-198 (*equal contribution)

> In this manuscript, I participated in the task design together with Born, J, Inostroza, M and Weber FD. I conducted the experiments, analyzed part of the behavioral data, and did all sleep EEG analyses. I structured and drafted the first manuscript together with Weber FD. Weber FD programmed the task, co-conduced two pilot behavioral experiments, analyzed the main behavioral data, and wrote the manuscript together with my input. Inostroza, M and Born, J conceptualized the study, interpreted the data and co-wrote the paper.

Wang, JY.*, Weber, FD.*, Zinke, K., Inostroza, M., & Born, J. (2017). More Effective Consolidation of Episodic Long-Term Memory in Children Than Adults - Unrelated to Sleep (accepted by *Child Development*) (*equal contribution)

> In this manuscript, I designed the task mainly with Weber FD and Born J, co-conducted the experiments with technical assistance from Eva Sitz, Sarah Fennel, Cristina Risueño and Yvonne Dages. I analyzed all the behavioral data, and scored all sleep EEG, I conceptualized and interpreted the data, and wrote the manuscript.
> Weber FD programed the task, analyzed part of the sleep EEG, co-interpreted the data for the final draft, and co-wrote the manuscript.
> Zinke, K was involved advising on experimental execution and co-edited the manuscript together with Inostroza, M., who conceptualized the original episodic memory study in adults.

Eva Sitz and Sarah Fennel assisted in collecting the children memory data. Cristina Risueño helped in data collection of the adult Pre-sleep group. And Yvonne Dages contributed for the adult Sleep group.

Wang, JY., Weber, FD., Zinke, K., Noack, H., & Born, J. Effects of sleep on word-pair memory in children – separating item and source memory aspects. (Submitted)

In this manuscript, I designed the task together with Weber FD, conducted the experiments with technical assistance from Eva Sitz and Sarah Fennel, Cristina Risueño and Yvonne Dages. I analyzed the behavioral data, and scored the sleep EEG, conceptualized and interpreted the data, and wrote the manuscript.

Weber FD programed the task, analyzed part of the sleep EEG, co-interpreted the data for the final draft, and co-wrote the manuscript.

Zinke, K gave advice on conducting the experiment and critically revised the manuscript together with Born, J. Noack, H adviced in interpretation of data.

Eva Sitz and Sarah Fennel assisted in collecting the children memory data. Cristina Risueño and Yvonne Dages each assisted in collection of one Adult group.

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Appendix

Study I:

Sleep's effect on episodic memory consolidation in adults

Research

Sleep benefits in parallel implicit and explicit measures of episodic memory

Frederik D. Weber,[1,3] Jing-Yi Wang,[1,3] Jan Born,[1,4] and Marion Inostroza[1,2]

[1]*Institute of Medical Psychology and Behavioral Neurobiology, University of Tübingen, 72076 Tübingen, Germany;* [2]*Departamento de Psicología, Universidad de Chile, 1058 Santiago de Chile, Chile*

Research in rats using preferences during exploration as a measure of memory has indicated that sleep is important for the consolidation of episodic-like memory, i.e., memory for an event bound into specific spatio-temporal context. How these findings relate to human episodic memory is unclear. We used spontaneous preferences during visual exploration and verbal recall as, respectively, implicit and explicit measures of memory, to study effects of sleep on episodic memory consolidation in humans. During encoding before 10-h retention intervals that covered nighttime sleep or daytime wakefulness, two groups of young adults were presented with two episodes that were 1-h apart. Each episode entailed a spatial configuration of four different faces in a 3×3 grid of locations. After the retention interval, implicit spatio-temporal recall performance was assessed by eye-tracking visual exploration of another configuration of four faces of which two were from the first and second episode, respectively; of the two faces one was presented at the same location as during encoding and the other at another location. Afterward explicit verbal recall was assessed. Measures of implicit and explicit episodic memory retention were positively correlated ($r = 0.57$, $P < 0.01$), and were both better after nighttime sleep than daytime wakefulness ($P < 0.05$). In the sleep group, implicit episodic memory recall was associated with increased fast spindles during nonrapid eye movement (NonREM) sleep ($r = 0.62$, $P < 0.05$). Together with concordant observations in rats our results indicate that consolidation of genuinely episodic memory benefits from sleep.

Originally, episodic memory has been defined with reference to stored "information about temporally dated episodes or events, and temporal–spatial relations between them" (Tulving 1983). Specific to episodic memory is that an experienced event upon its one-time occurrence becomes bound to the particular temporal and spatial context in which it occurred (Tulving 2002). However, apart from the binding of item memory into spatio-temporal context, the episodic memory concept originating from human research has also emphasized the dependence of episodic memory on autonoetic consciousness during recollection, which refers to a subjective awareness of the self as part of the remembered episode (Tulving 2001, 2002). Because examination of these subjective aspects of episodic memory appears to be suitable only for language-based approaches, research in animals has focused on the core features of episodic memory in terms of a memory for "what" (event) happened "where" (spatial location) and "when" (temporal order of events; Clayton and Dickinson 1998; Clayton et al. 2003), leaving unanswered the question to which extent this memory is truly episodic (Klein 2013; Pause et al. 2013).

There is now ample evidence that sleep benefits the consolidation of memory (Rasch and Born 2013). It has been proposed (Diekelmann and Born 2010) that sleep supports, in particular, the system consolidation of hippocampus-dependent memory which, in the classical view, is declarative memory and comprises episodic and semantic memories (Squire 1992; Diekelmann and Born 2010). According to this concept, slow wave sleep (SWS) promotes the neuronal reactivation of newly encoded hippocampal

memory representations and thereby not only strengthens them but also stimulates their redistribution to extrahippocampal networks serving as long-term store. Rapid eye movement (REM) sleep might add to consolidation by promoting synaptic consolidation processes which also would enhance nonhippocampal, e.g., procedural, types of memory (Diekelmann and Born 2010).

Although numerous studies in humans have demonstrated that sleep strengthens declarative memory, the effects of sleep on strictly episodic memory and its item-context binding features are less well investigated (Inostroza and Born 2013). There is some evidence that sleep preferentially strengthens context over item memory (Rauchs et al. 2004; Spencer et al. 2006; Lewis et al. 2011; van der Helm et al. 2011). However, others failed (Cairney et al. 2011), and none of these studies specifically examined the binding of an event into spatio-temporal context as a key feature underlying the formation of episodic memory. Notably, effects of sleep on the binding of item memory into spatio-temporal context have so far been directly examined only in one study in rats (Inostroza et al. 2013a). This study revealed sleep to, indeed, be necessary for upholding an integrative episodic-like representation. Rats which remained awake during the 80-min retention interval following encoding did not display any significant signs of episodic-like memory at retrieval testing, and in separate experiments these rats also forgot spatial and temporal context memory.

The present study followed two aims: First, based on the evidence in rats (Inostroza et al. 2013a), a supporting effect of sleep on core features of episodic memory—i.e., the binding of item memory into a spatio-temporal context—should be demonstrated in healthy humans. Second, we aimed at establishing a close link of the findings about the sleep-dependency of episodic-like

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memory in rats to human episodic memory. For this purpose, we adopted a task paradigm in humans that assessed episodic memory, like in rats, based on exploratory preferences (Kart-Teke et al. 2006). Whereas in rats exploratory locomotor behavior is typically used to assess episodic memory, in our human participants we used visual exploration. Importantly, in so doing we established a nonverbal, implicit measure of episodic-like memory in humans, which we tested for its correlation with truly explicit episodic memory recall.

Results

Implicit episodic memory

Analysis of variance (ANOVA) of normalized visual exploration time for the different items (faces) during the retrieval phase revealed different patterns depending on whether participants had slept or were awake during the retention interval between encoding and retrieval ($F_{(1,27)} = 5.52$, $P = 0.026$, for Displaced/Stationary [Spatial component] × Old/Recent [Temporal component] × Sleep/Wake [Condition]). Separate analyses of the Sleep and Wake groups indicated that only the Sleep group displayed significant episodic memory, i.e., a pattern of visual exploration that matched exploratory preferences in rodents with significant episodic-like memory (Li and Chao 2008; Inostroza et al. 2013a). The episodic nature of the expressed memory integrating temporal and spatial components manifests itself in the interaction between spatial and temporal components of the task ($F_{(1,14)} = 6.56$, $P = 0.023$, for Spatial component × Temporal component in a sub-ANOVA on the Sleep group) (Fig. 1B), i.e., a pattern that is primarily characterized by relatively shorter exploration time for the face that is both old–familiar and displaced than would be expected from adding up the spatial main effect (i.e., longer exploration for the displaced than stationary faces) and the temporal main effect (i.e., longer exploration for the old–familiar than recent–familiar faces). The Wake group did not display a significant pattern of visual exploration, i.e., no indication of episodic memory ($P > 0.36$, for Spatial component × Temporal component interaction). Post-hoc t-tests between the groups revealed greatest differences for the recent–familiar stationary item for which exploration time, on average, was shortest in the Sleep group (mean ± SEM, $431.9 ± 53.00$ msec) but longest in the Wake group ($763.57 ± 98.81$ msec, $t_{(27)} = -3.015$, $P = 0.006$, $d = 1.11$) (Fig. 1B). Spatial and Temporal component main effects were not significant ($P > 0.32$).

The presence of episodic memory in visual exploration patterns at retrieval

selectively in the Sleep group was confirmed using the "episodic binding" score in which basically the statistical Spatial component × Temporal component interaction term was used to specifically express the spatio-temporal binding underlying the formation of an episode (see Materials and Methods). Accordingly, significant spatio-temporal episodic binding was only observed in the Sleep group ($13.47 ± 5.25\%$, $t_{(14)} = 2.56$, $P = 0.023$, $d = 0.66$) but not in the Wake group ($-6.03\% ± 6.49\%$,
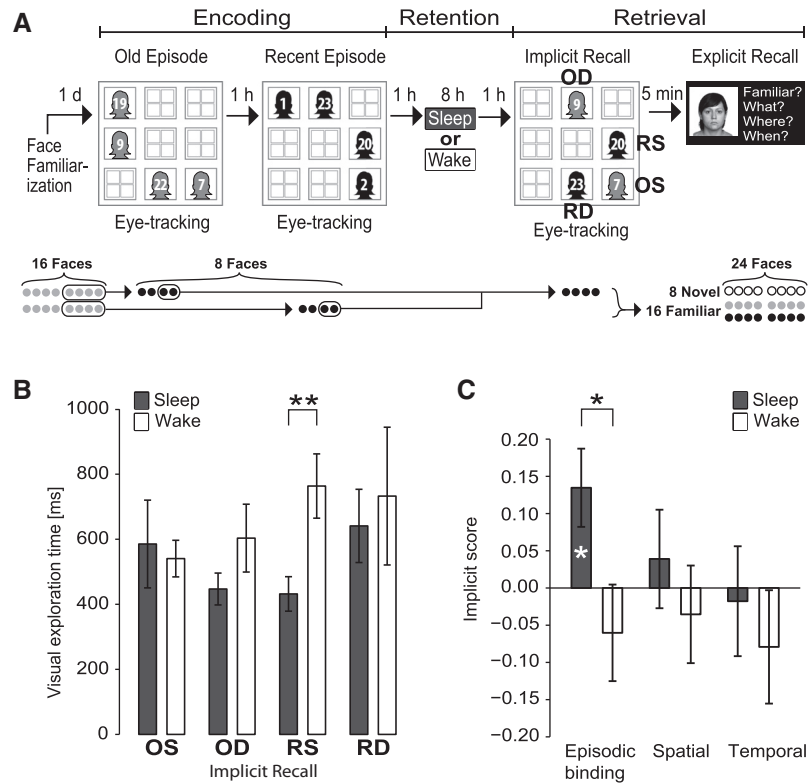
Figure 1. (*A*) Experimental design. Each session included an encoding phase, a retention interval, and a retrieval phase. The encoding phase comprised two episodes (Old Episode, Recent Episode) 1-h apart, each entailing a specific configuration of four individual faces in a 3 × 3 grid of locations. The subsequent 10-h retention interval contained either an 8-h interval of nighttime sleep (Sleep group) or daytime wakefulness (Wake group). The retrieval phase started with implicit recall which was assessed by eye-tracking visual exploration of another configuration of four faces. Two of these faces were from the first (Old) and second (Recent) episode, respectively, and of the two faces one was presented at the same location (Stationary) as during the episode and the other at another location (Displaced) resulting in four stimulus types: Old–familiar Stationary (OS), Old–familiar Displaced (OD), Recent–familiar Stationary (RS), and Recent–familiar Displaced (RD). Implicit recall was followed by explicit verbal recall testing. Face configurations were randomized across episodes and implicit recall. For the figure, individual faces are anonymized by ID-numbers representing one of the total set of 24 faces used in the task (first episode faces—black; second episode faces—gray; one gray-scaled example face illustrated for explicit recall). *Bottom* part illustrates faces used in the different experimental phases: in the face familiarization phase before the experiment proper, subjects were familiarized with 16 faces (gray circles), of which eight faces were used in the encoding phase of the episodic memory task, four in the old episode, and four in the recent episode. For implicit recall testing, four of the faces presented in the episodes of the encoding phase were used, two from each episode. During explicit recall testing 24 faces were presented, i.e., aside from the 16 familiarized faces (eight from episodes—black circles, eight not from the episodes but presented in the face familiarization phase—gray circles), and eight entirely novel faces (empty circles), which also allowed to discrimination between "face recognition" (novel vs. familiar) and "What" memory (familiar in episodes vs. familiar but not in episodes). (*B*) Mean (±SEM) visual exploration time for each stimulus type, and (*C*) "episodic binding" scores (indicating spatio-temporal binding in episodic memory) (see Materials and Methods) and separately measures of the spatial and temporal components in episodic memory during implicit recall testing, for the Sleep group (*n* = 15, filled bars) and the Wake group (*n* = 14, empty bars). Note, for clarity, absolute rather than normalized exploration time (i.e., exploration time divided by the total time of all looks on a face) is indicated. (*) $P < 0.05$, (**) $P < 0.01$, *above* bars for difference between Sleep and Wake groups, *within* bars (in panel C) for comparison with chance level (zero).
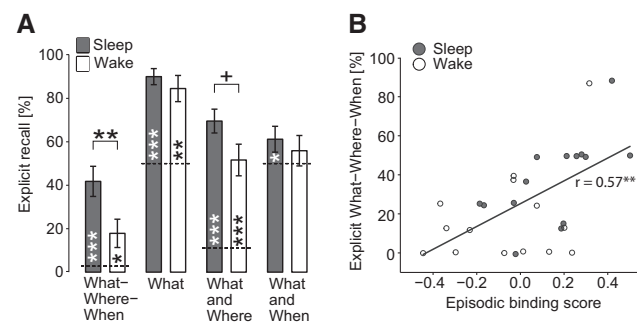
$t_{(13)} = -0.93$, $P = 0.37$), and the positive episodic binding score of the Sleep group also significantly differed from that of the Wake group ($t_{(27)} = 2.35$, $P = 0.026$, $d = 0.87$) (Fig. 1C). Scores formed separately for the spatial and temporal components of episodic memory (reflecting the statistical Spatial and Temporal main effects) failed to reach significance in both the Sleep and Wake groups and also did not differ between the groups (spatial score—Sleep, $3.90 \pm 6.62\%$; Wake, $-3.54 \pm 6.56\%$, $t_{(27)} = 0.80$, $P = 0.43$; temporal score—Sleep, $-1.78 \pm 7.38\%$; Wake, $-7.91 \pm 7.62\%$, $t_{(27)} = 0.58$, $P = 0.57$).

For both experimental groups, control analyses excluded any transition effects, i.e., exploration time was not influenced depending on whether or not during the encoding phase a certain grid location was occupied by an item in both episodes (Sleep, $t_{(13)} = -0.18$, $P = 0.86$; Wake, $t_{(12)} = -0.12$, $P = 0.91$; comparison between groups $t_{(25)} = -0.046$, $P = 0.96$, $d = 0.02$).

Explicit episodic memory

Explicit recall of episodic memory ("what–where–when" memory) was assessed after implicit retrieval measurement, and determined by the percentage of (all possible) faces that the participant correctly identified as occurring in one of the episodes ("what") and for which he also identified the correct episode ("when") and the location ("where") at which it occurred (see Materials and Methods). Explicit episodic recall was above chance in both groups (Sleep $P < 0.001$, Wake $P = 0.025$, Mann–Whitney test) but differed between the conditions, with performance being distinctly better in the Sleep than Wake group ($41.79 \pm 6.94\%$ vs. $17.86 \pm 6.51\%$, $P = 0.005$, $r = 0.51$) (Fig. 2A). Higher recall performance in the Sleep than Wake group was confirmed in an analysis restricted to faces that were not involved in implicit recall ($P < 0.022$) excluding a biasing influence of prior implicit recall testing. In an exploratory ANOVA, episodic memory recall did not differ between faces of the first and second episode ($P > 0.18$, for respective Episode main effect and Episode × Sleep/Wake interaction).

Separate analysis of the "what" component (number of faces correctly identified as belonging to one of the two episodes expressed as percentage of episode faces that were correctly recognized as familiar and belonging to one of the two episodes) revealed performance well above chance in both conditions

(Sleep, $90.0 \pm 3.70\%$, $P < 0.001$; Wake, $84.52\% \pm 6.03\%$, $P = 0.002$) but without a significant group difference ($P = 0.43$). The proportion of recalled faces (i.e., "what" component) for which the place was correctly recalled ("what and where") was above chance in both groups (Sleep, $69.55 \pm 5.48\%$; Wake, $51.62 \pm 7.28\%$; both $P < 0.001$), and was marginally (but not significantly) greater in the Sleep group ($P = 0.057$). The proportion of recalled faces for which the episode was correctly recalled ("What and When") was above chance only in the Sleep group ($61.19 \pm 5.95\%$, $P = 0.040$), but failed to reach significance in the Wake group ($55.90 \pm 7.01\%$, $P = 0.21$; for the difference between groups, $P = 0.57$). Interestingly, restricting the analysis of these items (for which "What and When" was correctly recalled) to only those for which the spatial component ("What–Where") was not correctly recalled yielded a significantly better recall for the Wake than the Sleep group ($12.71 \pm 3.91\%$ vs. $30.07 \pm 6.21\%$, $P = 0.016$) suggesting that temporal processing considered in isolation might be superior in the wake state. Finally, an overall analysis of faces correctly recognized as familiar, which included also those faces presented only during the familiarization phase, indicated that both the Sleep and Wake groups displayed close to ceiling object-recognition performance, with no differences between groups (Sleep, $91.67 \pm 3.64\%$; Wake, $94.64 \pm 2.61\%$, $P = 0.90$).

Correlation analyses

We calculated correlations between implicit and explicit measures of episodic memory and, for the Sleep group, between recall measures and sleep parameters. The implicit episodic binding score inferred from visual exploration was significantly correlated with explicit episodic memory recall ("What–Where–When") across both groups ($r = 0.57$, $P = 0.002$) (Fig. 2B), which was mainly driven by the sleep group (Sleep, $r = 0.63$, $P = 0.017$; Wake, $r = 0.21$, $P = 0.47$). There was no correlation between separate implicit and explicit scores of spatial or temporal components of episodic memory ($P > 0.30$).

The Sleep group displayed normal overnight sleep during the retention interval (see Table 1 for a summary of sleep parameters). Correlation analyses revealed a consistent pattern of moderate associations, in particular between NonREM sleep processes and implicit measures of episodic memory. Thus, the episodic binding score showed a positive correlation with time in NonREM sleep ($r = 0.68$, $P = 0.007$) and, in parallel, consistent correlations with centro-parietal (fast) spindle counts during NonREM sleep ($r = 0.62$, $P = 0.017$, for average spindle count across central and parietal electrodes) (Fig. 3). In further exploratory analyses, the separate temporal memory component score showed negative correlations with time in stage 2 NonREM sleep ($r = -0.71$, $P = 0.004$) and fast spindles counts over centro-parietal areas ($r = -0.57$, $P = 0.03$, for average count across electrodes). There were no consistent correlations with EEG power in the frequency ranges of interest with the exception of a negative correlation between "What" memory and NonREM EEG activity in the 0.5- to 8-Hz range that was highest for the delta band ($P < 0.0001$, $r = -0.88$). Explicit episodic memory recall did not show any significant correlation with sleep measures, and there was also no consistent association between memory measures and REM sleep parameters.

Memory for word-pair associates, vigilance, and mood

Between the experimental episodes of the encoding phase, participants learned two lists of word-pairs, i.e., a control task of declarative memory for which beneficial effects of sleep are well established (e.g., Plihal and Born 1997). As expected, recall of the word-pairs tested at the end of the retrieval phase was

Figure 2. (A) Mean ($\pm$ SEM) explicit recall of episodic "What–Where–When" memory and of subcomponents ("What," "What and Where," "What and When") during the retrieval phase for the Sleep ($n = 15$, filled bars) and Wake groups ($n = 14$, empty bars). ($^+$) $P < 0.1$, (*) $P < 0.05$, (**) $P < 0.01$, (***) $P < 0.001$, *above* bars for difference between groups, *within* bars for comparison with chance level (dotted line). (B) Pearson product–moment correlation between implicit episodic memory (Episodic binding score) and explicit episodic memory recall (What–Where–When), across the Sleep (filled circles) and Wake groups (empty circles, $n = 28$; data from one Sleep subject was excluded due to ceiling, 100%, explicit recall performance). (*) $P < 0.01$.

Table 1. Sleep parameters for experimental night of the Sleep group

Sleep stages	Time in minutes
TST	467.29 ± 6.01
Sleep onset	14.21 ± 3.52
Wake	15.93 ± 8.10
Stage 1	14.43 ± 2.82
Stage 2	239.29 ± 11.47
Stage 3	40.36 ± 2.19
Stage 4	55.25 ± 7.98
SWS latency	14.75 ± 1.50
SWS	95.61 ± 7.82
REM latency	113.46 ± 10.89
REM	98.64 ± 6.77

Data are means ± SEM, $n = 14$. Total sleep time (TST) and time in different sleep stages, sleep onset latency (with reference to lights off) and latency for slow wave sleep (SWS) and rapid eye movement (REM) sleep with reference to sleep onset.

significantly better in the Sleep than Wake group (99.21 ± 2.80% vs. 91.13 ± 2.67%, $t_{(27)} = 2.09$, $P = 0.047$, $d = 0.78$).

Vigilance was assessed by the Psychomotor Vigilance Task (PVT) before each episode during the encoding phase and before implicit recall during the retrieval phase, but did not differ between groups (Sleep vs. Wake: before first episode 291.35 ± 32.41 msec vs. 286.46 ± 20.34 msec; before second episode, 296.35 ± 21.72 msec vs. 291.92 ± 27.47 msec; before implicit recall, 291.73 ± 24.00 msec vs. 285.68 ± 23.11 msec; all $P > 0.50$). There were also no differences between groups in subjective sleepiness assessed by the Stanford Sleepiness Scale (SSS) (Sleep vs. Wake: before first episode 3.25 ± 1.14 vs. 2.84 ± 0.99; before second episode, 3.5 ± 1.17 vs. 2.92 ± 1.12; before implicit recall 2.50 ± 0.80 vs. 1.92 ± 0.76; all $P > 0.10$), and in mood, assessed by the Positive Affect Negative Affect Scale (PANAS) (Sleep vs. Wake group: before encoding phase, Positive Affect 24.43 ± 1.25 vs. 27.62 ± 1.8, $P > 0.40$, Negative Affect 12.93 ± 0.91 vs. 12.54 ± 0.55, $P > 0.76$; before retrieval phase, Positive Affect 26.16 ± 1.52 vs. 29.36 ± 1.64, $P > 0.23$, Negative Affect 12.54 ± 0.69 vs. 11.93 ± 0.35, $P > 0.66$).

Discussion

We report novel evidence indicating that sleep in humans strengthens the binding of an item memory into spatio-temporal context which is a core feature of episodic memory. Importantly, we assessed episodic memory implicitly by visual exploration times, and explicitly by verbal recall, and for both measures sleep compared to wakefulness produced a more than twofold increase in strength of episodic binding. Although explicit assessment suggests episodic binding is present after sleep and wakefulness, implicit assessment indicated above-chance episodic binding only if subjects slept after encoding. Compared with the distinct effect on episodic binding, sleep had only minor effects on separate implicit or explicit retrieval measures of "What," "Where," and "When" components of the encoded episodes. To immediately support a fresh episodic memory might represent a basic component of sleep's function in memory processing.

We established a novel task that allowed for assessing truly episodic memory in humans in both ways, i.e., implicitly, using visual exploration, and explicitly, using verbal recall. The task design originated from previous studies that employed behavioral exploration preferences to investigate episodic-like memory in rodents (Dere et al. 2006; Kart-Teke et al. 2006, 2007; DeVito and Eichenbaum 2010; Davis et al. 2013a,b; Inostroza et al. 2013a,b). The episodic nature of our task was further enhanced by using

unique faces that were presented in a unique spatio-temporal context. To reduce emotionality, we used faces with a neutral expression that, in addition, were familiarized before the experiment proper. Emotionality has been considered a feature inherent to episodic memory and, indeed, is a critical factor determining persistence of episodic memory (Libkuman et al. 2004; Dere et al. 2010; Pause et al. 2013). Nevertheless, we preferred to make the experienced episodes relatively neutral, because in this way visual exploration was expected to be determined predominantly by novelty, preventing that emotional aspects in the stimulus configuration masked memory-guided visual exploration.

Implicit memory was successfully indicated in the task by visual exploration time, specifically the time participants spent looking at a particular face at their first looks on a face, with the exploration times being characteristically enhanced when a face at retrieval testing is encountered in a conflicting spatio-temporal context relative to the previously encoded episodes. Thus, visual exploration time is longer for faces spatially displaced than for (stationary) faces presented at the same location as during encoding, and in parallel with temporal conflict, exploration time is longer for faces that belong to the older compared to the more recently encoded episode. A consistent exploration pattern for the faces with conflicting contexts indicates associated spatial and temporal memory. Crucially, episodic binding of an event together with spatial and temporal context components expresses itself in an interaction of spatial and temporal memory effects for a unique face, as derived from the exploration pattern, rather than in a mere additive effect of both components. This interaction between "when" and "where" effects on visual exploration time indicates that the gain in exploration time for faces that are both old and displaced (i.e., OD) is less than would be expected from adding up spatial and temporal main effects. It is this interaction expressing itself in a relatively reduced exploration of OD items that has been consistently revealed as an indicator of episodic-like memory in rodent studies (e.g., Kart-Teke et al. 2006, 2007; Inostroza et al. 2013a), and that provides a valid measure of an integrated rather than separated retrieval of spatial and temporal context, i.e., of contextual binding as a hallmark of episodic memory (Clayton et al. 2003, but see also Place et al. 2012).

Semantic memory can mask the assessment of episodic memory and, although our subjects were not instructed to learn anything, the encoding phase comprising three consecutive runs through each episode might have triggered semantic memory formation (Pause et al. 2013). However, simple models of familiarity-based recall, where trace strength is reflected in memory strength, would predict generally better recall performance for the recent than the older episode. This is not supported by our results as there were no significant differences in this direction
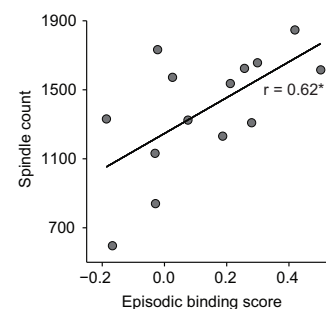
Figure 3. Pearson product–moment correlation in the Sleep group ($n = 14$) between fast sleep spindle counts during NonREM sleep and implicit episodic memory (Episodic binding score). (*) $P < 0.05$.

for any of the implicit or explicit memory measures. Explicit "what–where–when" memory was even better for the first than second episode in the Wake group, possibly reflecting proactive interference which was annulled by sleep (Abel and Bäuml 2013). Also, we controlled that our implicit measure of episodic memory is, indeed, item specific, i.e., it is only sensitive to a specific face ("what") and not to any face in a shared spatio-temporal context. This was indicated by calculating a "transition score" which excluded that mere spatial overlaps regarding the occupation of a grid location between the first and second episode significantly contributed to our episodic memory measure. Finally, implicit assessment of memory on only half of the items involved in each episode allowed us to control whether implicit recall biased subsequent assessment of explicit memory. Although such bias cannot be entirely ruled out, explicit "what–where–when" memory for the faces used in implicit recall testing showed no difference from memory for items that were not used.

Tulving (2002) considered episodic memory recall a capacity that involves the ability to "mentally time travel" and re-experience specific events, and thereby relies on a sense of self and conscious awareness that the experience occurred in the past. Although this definition reflects the phenomenological aspects of episodic memory in humans, it exclusively relies on the verbal report of subjective experiences. However, it prevents its investigation in animals. The absence of any objective behavioral measure for episodic memory is also not conducive to a rigorous scientific investigation of this kind of memory in humans (Allen and Fortin 2013). Here, this issue is addressed. A major advantage of our implicit memory measure is that it allows for examining episodic memory in nonverbal humans, i.e., infants, and also for comparisons across species. Although not specifically scoring for episodic binding, studies in rodents using exploration behavior in an analogous task design revealed an episodic-like pattern strikingly similar to that observed here for human visual exploration (Dere et al. 2006; Kart-Teke et al. 2006, 2007; Davis et al. 2013a,b). Furthermore, those studies showed that this episodic-like memory exploration pattern is crucially dependent on hippocampal function (DeVito and Eichenbaum 2010) and sleep (Inostroza et al. 2013a). The latter observation concurs with the present study where the episodic-like memory exploration pattern was also robustly expressed only in the participants of the Sleep group. Thus, the present findings constitute a strong link between sleep-dependency in human episodic memory and episodic-like memory in rodents. Pause et al. (2010) took an approach to explore episodic memory-like exploration patterns in humans comparable to ours. However, rather than on visual exploration they relied on explorative button presses as a nonverbal measure, and subjects were instructed to learn the episodes at encoding. At a recall test 24 h later, they found nearly significant spatial and temporal main effects, however no cues for episodic binding, suggesting that visual exploration might be more sensitive to preferences promoted by implicit memory than exploratory motor behaviors.

Sleep's function for declarative memory has been conceptualized to be an active system consolidation process rather than a passive protection against interference (Stickgold 2005; Diekelmann and Born 2010; Lewis and Durrant 2011; Inostroza and Born 2013; Rasch and Born 2013). According to this concept, an episode is encoded in both hippocampal and extrahippocampal networks, whereby the hippocampus preferentially encodes aspects binding items into their unique spatio-temporal context. During subsequent SWS, hippocampal portions of the representation are repeatedly reactivated to support an immediate strengthening of hippocampal traces and also redistribution toward preferential storage of information in extrahippocampal networks. This redistribution entails a transformation of represen-

tations toward more decontextualized schema-like representations (Marr 1971; Frankland and Bontempi 2005; Diekelmann and Born 2010; Winocur et al. 2010). The EEG slow oscillation and fast (12–15 Hz) spindles are considered hallmarks of the consolidation processes, in as much as the slow oscillation appears to synchronize hippocampal memory reactivations and accompanying sharp wave-ripples with the occurrence of spindles, thus allowing for the formation of spindle–ripple events as a mechanism supporting the transfer of reactivated memory information toward extrahippocampal circuitry (Mölle and Born 2011; Mölle et al. 2011). Consistent with this view, here we found a robust association between episodic memory recall and spindle counts during post-encoding NonREM sleep. Together with numerous previous studies showing similar correlations between sleep spindle activity and the retention of declarative as well as procedural memory (e.g., Gais et al. 2002; Tamaki et al. 2009; Barakat et al. 2011; Wilhelm et al. 2011; Rasch and Born 2013), this observation does not only underline the importance of spindles for memory processing in general but also points to a specific function of spindles in enhancing spatio-temporal integration in a memory, which might be conveyed via a direct impact on hippocampal networks (Clemens et al. 2007). The association with spindle activity occurring selectively for implicit rather than explicit episodic memory recall is difficult to explain in this context; it might be that the implicit measure is more sensitive, capturing variability in recall of hippocampal memories to a greater degree than the explicit recall measure.

Implicit but not explicit recall showing robust correlation with sleep spindles raises the question whether the two recall measures access the same representation, although via different retrieval pathways, or whether there exist distinct implicit and explicit representations that happen to correlate significantly because they encode for the same experience. That the wake group showed significant explicit episodic memory, but chance level performance for implicit episodic memory recall, could be taken as a hint for two different representations being accessed in the tests. This assumption would be further supported if implicit and explicit episodic memory underwent different transformations across sleep. However, as we did not assess recall before the retention interval, sleep-induced memory transformation could not be examined here. Nevertheless, the effects of sleep compared with wakefulness, being surprisingly in parallel for implicit and explicit measures of episodic memory, speak in favor for a common representation underlying both types of recall measures which, indeed, share the essential features of episodic experience, but the autonoetic consciousness that is produced only during explicit recollection.

Materials and Methods

Participants

Participants were healthy, nonsmoking, and native-speaking volunteers recruited from the campus of the University of Tübingen. They were randomly assigned to either the Sleep group ($n = 15$, nine men) aged (mean ± standard deviation) $23.72 ± 3.14$ yr, or the Wake group ($n = 14$, seven men, age = $24.25 ± 5.08$ yr). They had normal or corrected to normal vision and did not take any medication at the time of the experiments. They had a normal sleep–wake rhythm and were not on any night shifts during the 6 wk preceding the experimental session. Participants were instructed to keep their regular sleep schedule, abstain from caffeine- and alcohol-containing drinks for at least 3 d prior to and on the days of the experiments. Prior to the experiment proper, participants of the Sleep group spent one habituation night in the sleep laboratory. Subjects gave written informed consent before participating and the study was approved by the local ethics committee.

Design and procedures

The experiments were performed according to a between-groups design, including a Sleep group and a Wake group. For each group, the experiment consisted of an encoding phase followed by an ~10-h retention interval, followed by a retrieval phase (Fig. 1A). The encoding phase comprised the encoding of two experimental episodes, which were separated by 1 h. The retrieval phase included an implicit recall followed by an explicit recall of the materials learned in the encoding phase. For subjects of the Sleep group, the encoding phase took place between 8:15 pm and 10:45 pm, and the retrieval phase between 8 am and 9:30 am. Sixty minutes after the encoding phase, they went to bed (lights off) for an 8-h sleep period. The retrieval phase started 60 min after awakening. For the Wake group, the encoding phase took place between 7:15 am and 11:15 am, and the retrieval phase between 6:15 pm and 9:15 pm. During the wake interval, the subjects followed their usual activities outside the laboratory. They were not allowed to engage in stressful mental and physical activities. Activity during the retention interval was measured by a wristwatch (Actiwatch 2, Philips). Moreover, subjects provided a report about their activities during this time when they returned to the lab.

To confirm regular declarative memory benefits from sleep, a standard paired words associate learning task (two lists of 40 word-pairs) was used which in previous studies proved sensitive to the effects of sleep (Plihal and Born 1997; Ngo et al. 2013). Lists were learned to a criterion of 24 correctly recalled words (cued recall). In the encoding phase, one list was learned 20 min after presentation of the first episode of the episodic memory task, and the other 20 min after presentation of the second episode. In the retrieval phase, cued word recall was tested 10 min after episodic memory retrieval was completed.

Subjects were familiarized with the face stimuli used in the episodic memory task 1 d (in two cases 2 d) before the experiment proper, to avoid that the use of novel faces would distract the subject's attention from the spatio-temporal features of the task. To control for vigilance, before this familiarization phase as well as before the encoding phase, in between the two episodes, and before retrieval testing the Psychomotor Vigilance Test (PVT, 5 min) and the Stanford Sleeping Scale (SSS) were administered. To measure current mood, the Positive Affect Negative Affect Scale (PANAS) (Watson et al. 1988; Krohne et al. 1996) was given before the encoding and retrieval phases.

Episodic memory task

The encoding phase of the episodic memory task comprised the presentation of two episodes separated by 1 h during which subjects engaged in standardized activities (PVT, word-pair associates learning, filling in questionnaires, etc.). During each episode, participants were presented on a screen with a specific spatial configuration of four different faces arranged in a 3 × 3 grid of possible locations (with the center location of the grid left always empty) (Fig. 1A).

During the retrieval phase, implicit spatio-temporal recall performance was assessed by eye-tracking. For this purpose subjects were presented with another configuration of four faces used in the encoding phase (see below for the specific characteristics of this configuration to enable testing of implicit episodic memory). Participants were kept unaware during encoding and implicit recall testing that the task aimed at testing memory, but instead were told that attention was measured, and were instructed to attend to the presented stimulus configurations.

Spontaneous preference in visual exploration times was used as measure of memory, analogous to exploration preferences in rats; i.e., relatively longer fixation of a new than familiar face indicates item memory; relatively longer fixation of a familiar face presented at a new than at an old location indicates spatial memory, and relatively longer fixation of a face from the first than second episode indicates temporal memory (Ennaceur and Delacour 1988; Ennaceur and Meliani 1992; Mitchell and Laiacona 1998). Five minutes after implicit recall, explicit recall was measured by asking the subject successively whether a certain face occurred

in one of the two episodes, and if so in which episode and at what grid location it occurred.

Stimuli and stimulus presentation

Stimuli were 24 colored frontal images of natural female faces with neutral expression, placed on a white background (taken from the FACES database [Ebner et al. 2010]). Faces were randomized across subjects. Only female faces were used to reduce response variability (Penton-Voak et al. 1999).

Stimuli were presented on a monitor (ASUS Model VE248H, 24-in, 16:9, 1920 × 1080 px) and controlled using the software Presentation (Neurobehavioral Systems, version 15.1). Subjects sat in a comfortable position with an eye distance of ~60 cm in front of the screen, with their head leaning against an individually adjusted headrest on the back of the chair. To improve eye-tracking data during the episodic memory task, subjects were instructed not to move their head too much and to move only their eyes for visual exploration, but not head and neck.

During the familiarization phase before the experiment proper, subjects were presented with 16 faces (in random order) in five separate runs, separated by 30-sec breaks. Faces were presented one at a time with a 2-sec interstimulus interval. Immediately after stimulus onset, the face started moving smoothly for 1 sec (a minimum of 450 px) with the start and stop positions randomly chosen for each face presented on the screen. Then, the face rested for 7 sec on the stop position. One second before onset of each stimulus, a voice (from a speaker) signaled "look now" (German: "Schau mal"). Before the task, subjects were instructed to merely "pay attention" to the presented stimuli. The presentation scheme aimed to minimize any spatial interference with the face presentation during the episodic memory task using all available presentation space. To further avoid context interference, face familiarization took place in another room on another screen.

On the episodic memory task, the faces were presented on a 3 × 3 grid, with the grid location designed as windows of a house. Each episode started with indicating to the subject on the screen the episode ("1" or "2"); then the house with "closed windows" (gray framing without faces) was shown for 15 sec, and the subject was to fixate with his/her eyes a cross appearing in the center window (which was not used for face presentations). Once the fixation cross was fixated for 100 msec, it disappeared. During this time, instructions appeared on the screen, to concentrate and to explore in which window which face appeared (German: "Erkunden Sie, in welchen Fenstern welche Personen sind!"). Thereafter, with a delay of 50 msec, presentation of faces started as soon as the participant had fixated the fixation cross for 100 msec. Face presentation comprised two phases. In the "overview phase," all four face stimuli were shown on their respective location simultaneously for 15 sec. In the second "eye-trigger phase," the four faces were covered by "closed" windows and the specific face did not appear until the participant triggered its presentation by looking at the respective closed window area for at least 200 msec. The triggered face was then shown in the respective window. Presentation was discontinued as soon as the subject had looked at the respective stimulus area for a total of 7 sec (regardless of whether or not the gaze was intermittently directed away from the face location) which assured optimal control over the stimulus during encoding. Presentation of each face could be triggered only once, and the phase was completed when all four faces were discovered, i.e., triggered by the participant. During each episode the sequence of overview phase and eye-trigger phase was run three times in an identical manner.

Implicit recall testing consisted of only one overview phase. It involved a configuration of four familiar faces of which two were previously presented in the first ("old–familiar") and the other two in the second episode ("recent–familiar"). Of these, one was presented at the same location ("stationary") as during the respective encoding and the other was presented at another (new) location ("displaced") which was not occupied during the respective episode at encoding. This gives rise to four stimuli types: Old–familiar Stationary (OS), Old–familiar Displaced

(OD), Recent–familiar Stationary (RS), and Recent–familiar Displaced (RD). Thus, during implicit recall testing only faces that occurred during one of the two episodes of the encoding phase were presented, and no others. Although the face configuration during implicit recall testing was randomized, there were specific rules to position the stimuli that were mainly introduced to minimize potentially confounding effects on exploration time arising from the fact that a certain location could have been occupied by a face in just one episode or both episodes during encoding, and to exclude face-specificity of effects. Thus, the OS face was always on a location that had been occupied during both episodes, with the face from the second episode on this location not being used for implicit recall testing. The RS face was on a location that was empty during the first episode. The OD face was also on a location that was not occupied during the first episode, but was occupied by the face chosen for RD in the second episode. The RD face was then on a location that was not occupied during the second episode, but was occupied in the first episode with a face not used for implicit recall testing. Both episodes shared the location of the OS and another location presenting a face not presented at implicit recall testing. The center location of the grid was not used for face presentation to assure a similar distance of all faces from the center. Face presentation during implicit recall testing lasted between 30 and 60 sec, and within these margins was stopped (with a delay of 3 sec) as soon as looking time for each face was >3 sec for each of the four face areas.

Eye-tracking and implicit memory measurement

Eye movements were tracked using a remote system (Eye-Follower 2.0, interactive minds, tracking rate of 60 Hz on each eye). Eye-tracking was individually calibrated before each episode and before implicit recall testing. During calibration a colored filled circle traveled to one of 13 different calibration points, rested on each point until fixation of that position was detected by the eye-tracker, and then moved to the next calibration point. During calibration, the participant also received feedback about fixation performance accuracy. The calibration procedure was repeated until the assessed accuracy and precision reached a criterion of <0.6° deviation from five distributed test targets.

Eye-tracking analyses were restricted to the dominant eye as determined by the hole-in-the-card test (Dolman method) (Cheng et al. 2004). Fixations were detected using a 0.8° (30 px) visual angle gaze deviation threshold from the Euclidean centroid of the ongoing fixation and a minimum fixation duration of 100 msec (six samples), after removing artifacts (due to blinking, eye-occlusion, etc.). Visual exploration time of faces was measured based on "looks." A look on a face was considered the time the dominant eye was within the face's grid location, i.e., the gray frame surrounding a face, without leaving it. A look contained at least one fixation. Then, the visual exploration time spent on each face was determined by dividing the total time of all looks on a face by the number of looks on that face. Looks were sampled only up to the time point when all faces of the configuration had received at least one look.

Implicit memory

For determination of implicit memory, data from the first 30 sec of recordings were used. To reduce interindividual variability, visual exploration time for each face was normalized by dividing exploration time for the face by the sum of exploration time across all four faces (using absolute exploration times did not essentially change results reported here). Implicit episodic memory was defined by an "episodic binding" score assessing binding, i.e., integrated temporal and spatial aspects of an episode, as it manifests itself in the interaction between spatial exploration preference (i.e., longer exploration time for the "displaced" than "stationary" faces) and temporal exploration preference (i.e., longer exploration time for the "old" than "recent" faces). Specifically, based on corresponding studies of behavioral exploration in rodents (Kart-Teke et al. 2006, 2007; Inostroza et al. 2013a) this spatio-temporal interaction is expected to expresses itself in a gain of vi-

sual exploration time for an item that is both old and displaced (i.e., OD) which is distinctly smaller than would be expected from merely adding up spatial and temporal main effects. The episodic binding score was calculated as follows: $[(OS+RD) – (OD + RS)]/[OS + OD + RS + RD]$, where OS, OD, RS, and RD represent the visual exploration times for the old stationary, old displaced, recent stationary, and recent displaced faces, respectively. Because we hypothesized episodic memory consolidation produces longer visual exploration time for the OS and RD stimuli, the scores were defined such that values were positive for enhanced preference of those objects. Note, this score of episodic memory binding as defined by the interaction between spatial and temporal exploration preferences reflects the integrative assessment of both spatial and temporal information.

As an approach to infer spatial memory separate from temporal memory and vice versa, that is not reflected in the episodic binding score, we additionally calculated two different scores. The spatial score $[(OD + RD) – (OS + RS)]/(OS + OD + RS + RD)$ indicated enhanced preference of displaced (OD, RD) over stationary (OS, RS) faces; the temporal score $[(OS + OD) – (RS + RD)]/(OS + OD + RS + RD)$ indicated enhanced preference of faces from the first episode (OS, OD) over that of the second (RS, RD). To statistically confirm presence of memory, scores were compared with chance level (zero) using two-tailed one-sample t-tests.

To examine if exploration time was influenced depending on whether or not during the encoding phase a certain grid location was occupied by a face in both episodes, in control analyses we compared exploration time for faces in the second episode between those that shared (sharing) and those that did not share (nonsharing) the location with another face in the first episode, using the score (sharing – nonsharing)/(sharing + nonsharing). Significance (against zero) of this "transition" score indicated that a face of the second episode which shared its location with a face from the first episode was preferentially explored over second episode faces not sharing their location with a first episode face. The analysis was restricted to the first 15-sec interval of the second episode. Two participants (one Sleep, one Wake) were excluded from this analysis due to insufficient eye-tracking data on all four objects.

Explicit memory recall

For explicit recall testing, all 24 faces (eight presented during the episodes and during the familiarization phase, eight presented only during the familiarization phase, and eight completely novel) were presented consecutively (in random order) and the subject had to indicate (with no time limit, by mouse clicks on respective response text to questions presented on the screen) whether the face was new or familiar (object recognition); and if familiar, whether it occurred in the first or second episode (temporal "what–when" memory) or in none of the two episodes (i.e., was presented only during the familiarization phase), and whether it occurred in one of the episodes, at what grid location (spatial "what–where" memory). For the latter question, the grid was presented and the subject indicated the remembered location per mouse click. For each answer, confidence (0%–100% certainty) was rated immediately afterward. Subjects were trained on the recall procedure, using a dummy face, right before testing. In a final separate recall test, two grids were presented and the subject was asked to indicate which grid locations were occupied by faces during the first and second episode (spatial "where–when" memory).

Explicit episodic memory was determined by the percentage of faces that were correctly identified as occurring in one of the episodes (i.e., "what"), and for which the subject also correctly indicated the episode (i.e., "when") and the grid location (i.e., "where") it occurred, minus the locations for which the subject in the final separate recall test had forgotten that they were occupied with any face, with the number of episode faces correctly identified as familiar set to 100%. The chance level for this score was 2.78% (i.e., the chance to correctly recognize eight out of 16 familiar faces, one of two episodes, one of nine grid locations, for eight faces).

In addition, we separately assessed the "what" component of episodic memory as the percentage of episode faces that were correctly recognized as familiar and belonging to one of the two episodes, with the number of episode faces correctly identified as familiar set to 100%, The chance level for this score was 50.0% (eight of a total of 16 familiar faces). A general face recognition score was defined by the percentage of faces correctly identified as familiar (including those eight faces not used in the episodic memory task), with a chance level of 66.7% (16 familiar out of 24 faces). Finally, we calculated the percentage of correctly recognized faces for which also the episode ("what and when") or for which also the location ("what and where") was correctly recalled. The latter two scores were calculated additionally for only the cases where the "where" and "when" components, respectively, were not correctly remembered. The presence of explicit memory above chance level was assessed using one-tailed, one-sample *t*-tests or a nonparametric equivalent. Confidence ratings were not considered in the analyses.

Sleep recordings, EEG analyses, and spindles

To evaluate sleep in the Sleep group, polysomnographic recordings were performed, including EEG recordings from Fz, F3, F4, Pz, P3, P4, Cz, C3, C4 (according to the 10–20 system) with linked reference electrodes attached to the mastoids. An electrode at Fpz served as ground. Electrode impedance was <5 kohm. Additionally, the horizontal and vertical electrooculogram and electromyogram (from electrodes at the chin) were obtained. Signals were amplified (BrainAmp, Brain Product) and digitized at a sampling rate of 250 Hz. The EEG was filtered between 0.3 and 35 Hz. Sleep stages were scored offline in 30-sec epochs following standard criteria (Rechtschaffen and Kales 1968). For each subject, we determined sleep latency (with reference to lights off), total sleep time (starting with sleep onset), time spent in different sleep stages, i.e., wake, nonrapid eye movement (NonREM) sleep stages 1, 2, 3, and 4, and REM sleep (in minutes). Slow wave sleep (SWS) was defined by the sum of stage 3 and 4 sleep. Data from one Sleep subject was excluded due to corrupt EEG.

For a more fine-grained analysis of the EEG during sleep, power spectra were calculated using the Brain Vision Analyzer (version 2.0, Brain Products). Following removal of epochs contaminated by visually identified artifacts, Fast Fourier Transformations (0.061-Hz resolution) with a Hanning window was applied to a 10-sec data block which was moved in 5-sec steps in time during the respective sleep stage intervals. Average spectra were calculated across the time an individual spent in NonREM sleep (including stage 2, and SWS) and REM sleep, and also separately for the time spent in SWS and stage 2 sleep. Mean power was determined for the 0.5- to 4-Hz slow wave activity (SWA), the 0.5- to 1-Hz slow oscillation, 1- to 4-Hz delta, 4- to 8-Hz theta, 9- to 12-Hz slow spindle, and the 12- to 15-Hz fast spindle frequency bands.

In addition, to determine spindle density (per 30 sec) and counts, discrete slow and fast spindles were automatically identified during NonREM sleep (including stage 2 and SWS) using a custom-made algorithm (SpindleToolbox, version 1.1) as described previously in Wilhelm et al. (2011). Briefly, for each subject, the slow and fast spindle frequency peaks were visually identified from power spectra in the channels of interest (slow, 11.14 ± 0.16 Hz; fast, 13.37 ± 0.13 Hz); slow spindles were detected from fronto-central channels (Fz, F3, F4, Cz) and fast spindles from centro-parietal channels (Pz, P3, P4, Cz, C3, C4) according to their respective expected power maxima (Mölle et al. 2011). Then, the root mean square (RMS) of the EEG signal band-pass-filtered in the ± 1.5-Hz range around the detected spindle peak was calculated for subsequent 0.2-sec intervals separately for each EEG channel. A spindle was counted when the signal exceeded an individual amplitude threshold of 1.5 standard deviations from the mean RMS in a specific channel for 0.5–3 sec.

Statistical analyses

Statistical testing was done using [*R*] (64-bit Windows version 2.15.0) (R Core Team 2012). Values are given as mean ± SEMs.

Pre-tests involved Shapiro–Wilk's test for normality and for group tests, and Levene's test for homoscedasticity. To assess differences between Sleep and Wake groups, we used Student's *t*-test for equal variances and Welch's *t*-test with approximation to the degrees of freedom for unequal variances, when normality was assumed; otherwise we used nonparametric Mann–Whitney rank sum test with either exact *P*-values, or *P*-values that were continuity corrected in normal approximation. Cohen's *d* and Pearson's *r* were used to indicate effect size for parametric and nonparametric tests, respectively. Unless otherwise indicated *P*-values are reported uncorrected for multiple comparisons. The significance level was set to α < 0.05.

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Study II:

Comparison for sleep's influence on episodic memory consolidation in children and adults

Wang, JY.*, Weber, FD.*, Zinke, K., Inostroza, M., & Born, J.

More Effective Consolidation of Episodic Long-Term Memory in Children Than Adults -

Unrelated to Sleep

Jing-Yi Wang †[1,2], Frederik D. Weber †[1], Katharina Zinke[1], Marion Inostroza[1], Jan Born[1,3]

[1]Institute of Medical Psychology and Behavioral Neurobiology, University of Tübingen,

Tübingen, Germany

[2]Graduate School of Neural and Behavioural Sciences, University of Tübingen, Tübingen,

Germany

[3]Werner Reichardt Center for Integrative Neuroscience, University of Tübingen, Tübingen,

Germany

† Equal contribution of authors

Author Note

contributed equally.

Correspondence concerning this article should be addressed to Jan Born, Department of Medical Psychology and Behavioral Neurobiology, University of Tübingen, Otfried-Müller-Str. 25, 72076 Tübingen, Germany. Email: jan.born@uni-tuebingen.de. Tel: +49(0)7071-29-88924. Fax: +49(0)7071-29-25016

Abstract

Abilities to encode and remember events in their spatiotemporal context (episodic memory) rely on brain regions that mature late during childhood and are supported by sleep. We compared the temporal dynamics of episodic memory formation and the role of sleep in this process between 62 children (8-12yrs) and 57 adults (18-37yrs). Subjects recalled "what-where-when" memories after a short, 1-hour retention interval, or after a long, 10.5-hour interval either containing nocturnal sleep or daytime wakefulness. Although children showed diminished recall of episodes after 1 hour, possibly resulting from inferior encoding, unlike adults, they showed no further decrease in recall after 10.5 hours. In both age groups, episodic memory benefitted from sleep. However, children's more effective offline retention was unrelated to sleep.

Keywords: electroencephalography, declarative memory

More Effective Consolidation of Episodic Long-Term Memory in Children Than

Adults - Unrelated to Sleep

Episodic memory is "an information processing system that receives and stores information about temporally dated episodes or events, and about the temporal-spatial relations among these events." (Tulving, 1972). The episodic memory system essentially relies on the hippocampus, which, together with the prefrontal cortex and posterior parietal cortex, forms representations for unique events that occur in a distinct spatio-temporal context (Cabeza Ciaramelli, Olson, & Moscovitch, 2008; Preston & Eichenbaum 2013), e.g. a memory for what exactly happened at a specific time and place in one's life. In the adult brain, episodic memory representations are thought to form the basis for the formation of more semantic and schema-like representations that lack contextual detail, and can be accessed independently of the hippocampus (Dudai, Karni, & Born, 2015; Winocur, Moscovitch, & Bontempi, 2010). Sleep is an integral part of this consolidation process, as newly encoded neural episodic representations are reactivated in hippocampal and cortical networks during slow wave sleep (SWS) (O'Neill, Pleydell-Bouverie, Dupret, & Csicsvari, 2010; Rasch & Born, 2013). This strengthens both the episodic representation as well as its transformation into less context dependent semantic memory (Inostroza & Born, 2013; Oyanedel et al., 2014; Sweegers & Talamini, 2014; Weber, Wang, Born, & Inostroza, 2014).

The episodic memory system shows developmental trajectories from early childhood well into adolescence. Explicit recall of episodic memory seems to be functioning in a rudimentary way at the age of 3 to 4 years (Hayne & Imuta, 2011). The ability to fully

organize the experienced events in the context of when and where they occurred then improves throughout the first decade of life and even beyond (Bauer et al., 2012; Picard, Cousin, Guillery-Girard, Eustache, & Piolino, 2012; Yim, Dennis, & Sloutsky, 2013). A recent study suggests that memory for where events occurred reaches adult performance by 9½ years, whereas memory for when events occurred continuously improves into adulthood (Lee, Wendelken, Bunge, & Ghetti, 2016). This is in contrast to a previous study by Guillery-Girard et al. (2013) which also confirms the age between 9 and 10 years as a critical step in episodic development, but rather suggests that memory for where events occur improves up until adulthood. This slow development appears to be partly due to the protracted maturation of the brain structures involved in episodic memory formation (Ghetti & Bunge, 2012; Gogtay et al., 2006; Seress & Abraham, 2008). For example, myelinization in the prefrontal cortex is not completed until late adolescence (Teffer & Semendeferi, 2012). In contrast to structural immaturity of memory systems, however, children's sleep is marked by an increase of electroencephalographic activity that is related to memory consolidation: children sleeplonger and deeper, with increased proportions of SWS (Mindell, Owens, & Carskadon, 1999; Ohayon, Carskadon, Guilleminault, & Vitiello, 2004). Specifically, EEG slow-wave activity (SWA, 0.5–4 Hz), which is a hallmark of SWS, causally contributes to the consolidation of hippocampus-dependent memory (Marshall, Helgadóttir, Mölle, & Born, 2006; Ngo, Martinetz, Born, & Mölle, 2013). SWA reaches its plateau during preadolescence at around 9–11 years of age, i.e., an age when also hippocampus-dependent memory function appears to reach a first maximum (Feinberg, Higgins, Khaw, & Campbell, 2006; Huber &

Born, 2014). Indeed, 8 to 11-year-old children learning an implicit motor sequence task showed distinctly higher gains in explicit sequence knowledge after sleep than adults (Wilhelm et al., 2013). This suggests that processing of hippocampal memory during post-learning sleep is enhanced at this age. However, the effects of sleep on genuinely episodic memory, i.e., a memory for "what" happened "where" and "when", to our knowledge, have not been directly compared between children and adults.

Against this backdrop, here, we examined how recall of an episodic memory developed over time in 8–12 years old children and in adult controls, also taking into account the role of sleep. We used an episodic memory task that enabled integrated as well as separate testing of what, where, and when aspects of experienced episodes in both age groups (Weber et al., 2014). Memory was tested either after a short 1-hour retention interval or after a long 10.5-hour retention interval, with the latter including either a period of nocturnal sleep or daytime wakefulness. Because brain structures that serve the encoding of episodic memory are not completely mature in children, we expected children to show diminished recall performance compared to adults after short and long retention intervals not involving sleep. By contrast, the deeper SWS in children was expected to enhance consolidation of episodic memory over the long retention interval including sleep and reduce further forgetting to a higher degree than in adults.

Methods

Participants

Sixty-two children (age range 8–12 years), and 57 adults (18–37 years, only one

above 30 years) from the same mid-sized city in southern Germany participated in the

experiments in one of three retention conditions (Pre-Sleep, Sleep, and Wake). Groups of

children and adults in the three conditions were matched on age and general cognitive

performance (assessed by a computerized version of a forward digit span task, Blackburn,

2011). Furthermore, there were no differences in parent- or self-reported habitual sleep length

or bedtime between conditions. Data collection took place between 2012 and 2015.

Children were recruited from local schools and adults by advertisements distributed at the

university campus. All participants were native German speakers, healthy and had normal or

corrected to normal vision. They reported not to nap habitually or have any sleep disorders

(e.g., sleep apnea, irregular sleep, insomnia etc.), and did not take any medication at the time

of the experiments. Post-hoc analysis of sleep data in the Sleep participants indicated no

further sleep disorders (e.g. epilepsy, bed wetting, sleep walking, night terrors etc.). A general

questionnaire (containing questions like "Does your child have any known chronic illness?",

"Does your child (or someone in the family) have an attention deficit disorder?") that parents

answered for their children excluded known neurological and psychiatric disorders (such as

attention deficit hyperactivity disorder, dyslexia, autism, and epilepsy). Participants had an

age-appropriate sleep–wake rhythm and (adults) were not on any night shifts during the 6

weeks preceding the experimental session. Participants were instructed to keep a regular

sleep schedule, abstain from caffeine- and alcohol-containing drinks for at least 3 days before

and on the days of the experiments. Children kept sleep diaries for one week before the

experiments started. Adult participants and children's caregivers gave written informed

consent prior to the experiments. The study protocol was approved by the local ethics committee.

Design and procedures

The study followed a 2 (Age groups) × 3 (Retention conditions) between groups design. Accordingly, children and adults were allocated to one of 3 Retention conditions: with memory being tested either after a short 1-hour retention interval in the early evening (i.e., Pre-sleep groups: children: $n = 20$, 8 males, age: 9.86 ± 0.30 years; adults: $n = 18$, 10 males, 23.13 ± 0.82 years), or after a long 10.5-hour interval including nocturnal sleep (Sleep groups: children: $n = 21$, 10 males, 9.86 ± 0.25 years; adults: $n = 25$, 14 males, 23.53 ± 0.25 years) or daytime wakefulness (Wake groups: children: $n = 21$, 12 males, 9.95 ± 0.25 years; adults: $n = 14$, 7 males, 24.25 ± 1.36 years). Adult Sleep and Wake groups included a subsample of subjects from a previously reported study (Weber et al., 2014), with one group not extended for replication (Wake, $n = 14$). Assignment of subjects to the different retention conditions was random except that, for children, practical and other issues were also considered (e.g., driving distance between home and lab, school schedule, siblings were allocated to different groups). Experiments with children were mostly conducted on holidays and weekends.

The experimental procedure for all groups (illustrated in Figure 1) consisted of an encoding phase comprising the learning of an episodic memory task, a retention interval and a retrieval phase where the memory had to be recalled. In the Pre-sleep groups, the retention interval was short, i.e., 1 hour, whereas in the Sleep and Wake groups this retention interval was longer covering an interval of ~10.5 hours, including a nocturnal period of sleep or a

daytime period of continuous wakefulness, respectively. Learning and retrieval phases were timed individually with reference to the reported habitual sleep schedules ensuring appropriate testing times for children and adults, alike, while strictly keeping to the 1-hour or 10.5-hour retention interval schedule. In an additional familiarization phase taking place ~24 hours before the encoding phase, all participants were familiarized with the face stimuli of the episodic memory task. Participants of the Sleep groups also spent the night following the familiarization phase in the lab to habituate to sleeping under laboratory conditions.

Participants of the Sleep groups arrived at the lab about 3 hours before their usual bedtime. Following preparation for the EEG and polysomnographic recordings, they performed the episodic memory task (encoding, children between 6:00–8:00 pm, adults between 7:00–10:45 pm), and then prepared for sleep in the lab (lights off in children about 30 minutes, in adults about 60 minutes, after the encoding phase). Time in bed was 9.5 hours for children and 8 hours for adults. The retrieval phase started 30 minutes after waking (from stage 2 or 1 NonREM sleep) for children and 60 minutes after waking for adults. The slight difference in the timing of sleep between children and adults was introduced to keep the overall length of the retention interval comparable in both age groups, and to simultaneously account for the fact that children sleep longer than adults.

In the Wake groups, the encoding phase took place between 7:00–9:00 am in children, and between 7:15–11:15 am in adults. Afterwards, the participants followed their normal daily routine but were instructed to avoid stressful mental and physical activities. They were not allowed to take a nap, which was controlled by actigraphy (Actiwatch 2, Philips, The

Netherlands). Adherence to the instructions was controlled (by interview) when the

participants came back to the lab for the retrieval phase (approximately 7:00 pm in children,

between 6:15–9:15 pm in adults). In Pre-sleep participants, the encoding phase was

scheduled approximately 3.5 hours before the usual sleep time (between 4:00–6:00 pm in

children, between 7:00–8:30 pm in adults), and the retrieval phase 1 hours later. Participants

in the Wake and Pre-sleep groups did not sleep in the lab after the retrieval and went home.

Breaks during the experiments were filled with card games with the experimenter or a

puzzle-like game on the computer (www.snood.com, like it has been done in previous studies,

e.g., Feld, Weis, & Born, 2016). Short breaks were introduced if needed or requested by the

participants or to care for the age-appropriate bodily needs (e.g., drink water, go to toilet) and

to keep motivation up (e.g., experimenter interaction). To control for effects on executive

functions (i.e. the cognitive control of behavior) and possibly confounding effects of

sleepiness, vigilance was assessed using reaction time performance during a 5-minute

interval on the Psychomotor Vigilance Task (PVT, Roach et al., 2006), and sleepiness was

assessed by self-report using the Stanford Sleepiness Scale (SSS, Hoddes, Dement, &

Zarcone, 1972). Assessment of SSS and PVT were introduced to the participants in the

familiarization phase prior to the experiment proper and applied before encoding of each of

the two episodes of the episodic memory task, and again before retrieval testing.

Episodic memory task

The episodic memory task was adopted from a previous study (Weber et al., 2014;

Figure 1). The task stimuli included a total of 24 female faces, 16 of which the subjects had

studied in the familiarization phase 24 hours before the experiment proper. Of these 16 faces, 8 were used for the encoding phase. The encoding phase of the task comprised the presentation of two episodes separated by 1 hour, during which the participants engaged in standardized activities (including performance on control tasks, see above). Each episode consisted of the presentation (on a PC monitor) of a specific spatial configuration of 4 different faces arranged in a 3×3 grid of possible locations (with the center location of the grid always empty). In fact, each face appeared in a certain window of a house, and the participant was instructed that "in this game", he or she "needs to explore which person is behind which window of this house" (incidental learning instructions). Participants were kept unaware that the task measured memory, but were instructed to keep focused on the task as trained before the experiment proper. Eye movements were tracked (Eye-Follower, 2.0, interactive minds, tracking rate 60 Hz on each eye) for closed-loop control of the face presentations during the episodes to automatically control for encoding time and to handle the loss of attention without experimenter interference.

Each episode started by indicating (on the screen and for children, additionally, spoken via loudspeakers) the episode ("Game 1" or "Game 2"); then the house was shown with closed windows for 15 s, and the participant was instructed to fixate on a cross appearing in the center window with his or her eyes. Once the cross was fixated for 100 ms, it disappeared, and instructions were given (on the screen and via loudspeakers) to explore which face appeared in which window. Thereafter, the presentation of faces started. First, in an "overview phase", all 4 faces were shown simultaneously at their respective location for

15 s. Then, in the "eye-trigger phase", the 4 faces were masked by closed windows, and a specific face reappeared after the participant had triggered its presentation by looking at the respective closed window area for at least 200 ms. The presentation of a face in the specific window was discontinued as soon as the participant had looked at the respective area for a total of 7 s (regardless of whether or not the gaze was intermittently directed away). This assured that the face presentation time was constant for all subjects. Presentation of each face could be triggered only once, and the eye-trigger phase was completed whenever all 4 faces were discovered and shown for 7 s, or after 240 s. During each episode, the sequence of overview phase and eye-trigger phase was run 3 times in an identical manner. On altogether 9 runs children failed to trigger all 4 faces in all eye-trigger phases. Data of 1 child was excluded from analysis because he failed to trigger the 4 faces on all runs. The encoding phase lasted about 4–12 minutes, depending on how fast participants discovered faces in the eye-trigger phases.

For explicit recall testing during the retrieval phase, all 24 faces (8 presented during the episodes and also during the familiarization phase, 8 presented only during the familiarization phase, and 8 completely novel) were presented consecutively in random order and the participant had to indicate via mouse clicks (i) whether a face was new or familiar (object recognition); (ii) if familiar, whether it occurred in one of the episodes or not ("what" memory, i.e., was presented only during the familiarization phase the day before) and (iii) if it was the first or second episode (temporal "what–when" memory). (iv) If the participant indicated that the face occurred in one of the episodes, he or she should indicate at which

window location the face occurred (spatial "what–where" memory). In a final separate test, subjects were presented with a grid and asked to indicate which grid locations were occupied by faces during the first and second episode (object unspecific spatio-temporal "where–when" memory). (Adults additionally gave confidence ratings immediately after each answer, which were not analyzed here). Participants were trained on the recall procedures, using a dummy face, right before testing. There was no time limit for answering. Children reported their answer orally and the experimenter clicked the respective answers accordingly. To prevent biasing, the experimenter was blind to which faces and locations were used for an individual participant during the encoding phase. For standardization purposes, all instructions and questions were recorded and read to the children by a computer generated female voice.

Explicit recall testing was preceded by a short implicit memory test using eye tracking, for which the house and two faces from each episode (on either changed or unchanged locations) were presented for up to 60 s. Respective data will be reported elsewhere. Implicit memory testing was followed by a 5-minute relaxation pause before continuing with the explicit recall to reduce cognitive load and avoid interference with the explicit recall procedure. To exclude that this implicit memory test affected explicit recall, the explicit recall was also analyzed for only those faces not used for implicit recall testing. These analyses confirmed virtually all results reported here for the explicit recall of all face stimuli on the episodic memory task and that implicit testing did not affect children more than adults.

Stimuli and familiarization. Twenty-four colored frontal images of natural female faces with neutral expression (taken from the FACES database, Ebner, Riediger, &

Lindenberger, 2010), placed on a white background, were used as stimuli. Faces were randomized across subjects. Only female faces were used throughout the whole task to reduce response variability (Penton-Voak et al., 1999). Stimuli were presented on a monitor (ASUS Model VE248H, 24-in, 16:9, 1920 × 1080 px) and controlled using the software Presentation (Neurobehavioral Systems, version 15.1). Participants sat in a comfortable position with an eye distance of ~ 60 cm from the screen, with their head leaning against the back of the chair for stability. To improve eye-tracking data, subjects were instructed not to move their head but use only their eyes for visual exploration.

For familiarizing the subjects with 16 faces (on the day before the experiment proper), the faces were presented one at a time with a 2-s interstimulus interval. Immediately after stimulus onset, the face started moving smoothly for 1 s (a minimum of 450 px) with the start and stop positions randomly chosen for each face presented on the screen. Then, the face stopped moving for 7 s. Before the onset of each stimulus, a voice signaled, "Look!". The familiarization phase comprised 5 runs of all faces, with intermittent breaks of variable length (0.5–10 minutes). The subjects were instructed to focus on looking at the stimuli.

Memory scores. Episodic memory, i.e., "What–Where–When" memory, was determined by the percentage of the faces that were correctly identified as occurring in one of the episodes (i.e. "What"), and for which the subject also correctly indicated the episode (i.e., "What–When"), and the grid location (i.e., "What–Where") it occurred, minus the locations for which the subject had forgotten that they were occupied with any face in the final separate recall test (false "Where-When" memory), with the number of episode faces correctly

identified as familiar set to 100%. The subtraction of false "Where-When" memory was done to exclude that previously correctly recalled face locations were guessed or attributed to not integrated separate "What-Where" memory. Thus, the chance level of "What–Where–When" memory was 2.78% (i.e., the possibility to correctly recall 8 out of 16 familiar faces, in 1 out of 2 episodes, and in 1 out of 9 grid locations). The "What" component of episodic memory was defined by the percentage of faces that were correctly recognized as occurring in either episode, with the number of episode faces that were identified as familiar set to 100%. The chance level for the "What" memory was 50% (8 of 16 familiar faces). The "What–Where" and "What–When" components were calculated as the percentage of the faces with correct "What" memory, for which the location and the episode, respectively, were correctly identified. The chance level for the "What–Where" memory was 11.11% (1 out of 9 locations) and 50% (1 out of 2 episodes) for "What–When" memory.

Finally, a score for general face recognition was calculated based on the percentage of faces correctly identified as familiar (including those 8 faces not used in the episodic memory task), with a chance level of 66.7% (16 familiar faces out of 24).

Sleep recordings and EEG analysis

Sleep group participants received polysomnographic recordings including EEG recordings from Fz, F3, F4, Cz, C3, C4, Pz, P3, P4 electrode sites (International 10–20 system, reference: linked electrodes at the mastoids, ground at Fpz), electromyography (EMG) recordings from electrodes placed at each musculus mentalis, and electrooculography (EOG) recordings from electrodes around the eyes. In children, two EOG electrodes were placed 1

cm above the left outer canthus and 1 cm below the right outer canthus, respectively, whereas in adults four electrodes were placed 1 cm right to the right outer cantus, 1 cm left to the left outer cantus, and 1 cm each above and below the center of the right eye. Electrode impedances were kept below 5 kOhm. Signals were amplified (BrainAmp, Brain Products, Gilching, Germany), digitized (sampling rate >250 Hz) and filtered (EEG and EOG 0.3–35 Hz, EMG 10–100 Hz). Sleep stages were scored offline by two experienced raters according to standard criteria (Rechtschaffen & Kales, 1968). For each subject, total sleep time (TST, starting with sleep onset), time spent in sleep stages: stage 1, 2, SWS (the sum of stage 3 and stage 4), non-rapid eye movement (NonREM) sleep (sum of stage 2 and SWS), REM sleep and wakefulness, their proportion to TST, as well as SWS and REM sleep latencies were determined. Sleep onset was defined with reference to lights off by the first occurrence of stage 1-sleep epoch followed by stage 2-sleep.

For a more fine-grained analysis, power spectral analyses were performed on the NonREM sleep EEG to determine mean power density in the following frequency bands: slow-wave activity (0.5–4 Hz), theta (4–8 Hz), spindles (9–15 Hz), slow spindles (9–12 Hz) and fast spindles (12–15 Hz). Furthermore, slow oscillations and spindles during NonREM sleep were analyzed according to previously published algorithms (Mölle, Marshall, Gais, & Born, 2002). For each individual and channel, the number of slow oscillations, their density (per minute NonREM sleep), mean amplitude, and slope, as well as absolute spindle counts, spindle density (per minute NonREM and SWS for fast and slow spindles, respectively), mean amplitude, average oscillatory frequency and duration were calculated (see Supporting

Information for a detailed description of these sleep EEG analyses).

Data reduction and statistical analysis

Episodic memory performance data from one of the Pre-sleep and one of the Sleep children had to be excluded because of technical problems during encoding. One Wake child was excluded because he took a nap during the retention interval. Sleep data from three children and one adult were discarded due to technical artifacts. Thus, the sample available for the episodic memory performance consisted of 59 children (Pre-Sleep: n = 19; Sleep: n = 20; Wake: n = 20) and 57 adults (Pre-Sleep: n = 18; Sleep: n = 25; Wake: n = 14). For the sleep group, sleep data was available for 24 adults and 18 children with only 17 children providing complete data for the correlational analyses with episodic memory performance. Statistical analysis was done using [R] (Mac OS X version 1.7.1, R Core Team, 2012). Means ± SEM are reported. Normality and homogeneity were pre-tested using Shapiro-Wilk´s test and Levene´s test, respectively. Analyses were based on global 2 (Age groups) × 3 (Retention conditions) analysis of variance (ANOVA), in which, children and adults represented the Age groups, and Pre-sleep, Sleep, and Wake groups represented the retention conditions. Significant interactions in the global ANOVA were followed up by two 2 × 2 sub-ANOVA. One was designed to examine the effects of sleep vs. wakefulness and, aside from the Age group factor, included a Sleep vs. wake factor, representing the Sleep and Wake groups. The other sub-ANOVA was designed to examine the dynamics of episodic memory, across the short 1-hour (Pre-sleep) recall and the longer-term recall after Sleep, aside from the Age group factor. *Post hoc* tests followed significant ANOVA effects, including Student's *t*-test or,

if variances were unequal, Welch's *t*-test with an approximation for the degrees of freedom, if normality of both samples was not violated. Otherwise, we used nonparametric Mann-Whitney *U* test (Wilcoxon rank-sum test). Cohen's *d* and Pearson's *r* were used to indicate the size of central effects for parametric and nonparametric tests, respectively. For correlational analyses, Pearson product-moment and Spearman´s rank correlation were used, respectively. Because these analyses were of exploratory nature and no possible association should be missed, we did not correct the level of significance for multiple testing in these analyses (which nevertheless did not yield any significant correlation). The significance level was set to 0.05.

Results

Episodic memory

Explicit recall of episodic memory ("What–Where–When") was above chance in all six experimental groups (children Pre-sleep: $t_{(18)} = 4.41$, $p < .001$, Sleep: $t_{(19)} = 7.72$, $p < .001$, Wake: $p < .001$, adults Pre-sleep: $t_{(17)} = 10.37$, $p < .001$, Sleep: $t_{(24)} = 7.13$, $p < .001$, Wake: $p = .025$, *t*-test and Mann–Whitney *U* test, respectively, one-sided). Children and adults showed different dynamics of episodic memory recall across the short 1-hour (Pre-sleep) retention interval and the longer (sleep and wake) retention intervals ($F_{(2,110)} = 6.54$, $p = .002$, for Age group × Retention condition in a global ANOVA, see Figure 1C, for a summary of results including pairwise statistical comparisons). Both children and adults showed better episodic memory recall after sleep than after a comparable retention period of wakefulness ($F_{(1, 75)} = 11.74$, $p < .001$, for Sleep vs. wake main effect in a 2 (Age groups) ×

2 (Sleep vs. wake) sub-ANOVA), with the average magnitude of this sleep-dependent recall enhancement being closely comparable in the two age groups ($p = .29$, for Age $\times$ Sleep vs. wake interaction; What–Where–When recall rates, children - Sleep: 34.23 $\pm$ 4.07%, Wake: 21.34 $\pm$ 4.63%, $W = 278.5$, $p = .03$, $r = .4$, and adults - Sleep: 42.12 $\pm$ 5.52%, Wake: 17.86 $\pm$ 6.51, W = 275.5, $p = .002$, r = .55).

Notably, the sleep effect in the two age groups rode on quite different pre-sleep performance levels. Whereas the adult Pre-sleep group showed, as expected, high rates of episodic memory performance after a short 1-hour retention interval, respective recall rates of the Pre-sleep children were on average less than half of those of the adults (26.24 $\pm$ 5.32 % vs. 61.61 $\pm$ 5.67 %, in the Pre-sleep adults). With reference to their high recall performance after the short 1-hour interval, adults forgot episodic memory across the longer Sleep interval (and even more so across the Wake interval). By contrast, the children showed virtually no further decrease in recall (i.e., forgetting) across the Sleep interval, but on a descriptive level episodic memory recall was even increased after Sleep compared to the Pre-sleep condition. This pattern was statistically confirmed in a 2 (Age group) $\times$ 2 (Pre-sleep vs. sleep) sub-ANOVA, revealing a significant Age group $\times$ Pre-sleep vs. sleep interaction ($F_{(1, 78)} = 6.72$, $p = .01$). *Post hoc* *t*-tests confirmed that this effect was solely driven by the large difference between age groups in "What–Where–When" recall in the Pre-sleep condition ($t_{(34.71)} = 4.54$, $p < .001$, $d = 1.50$), whereas after sleep, episodic recall was comparable between the age groups ($t_{(41.65)} = 1.15$, $p = .26$). Also, the tests confirmed significant forgetting in the adults from the 1-hour Pre-sleep recall to the longer-term recall after Sleep (t

$_{(39.41)}$ = 2.46, $p < .02$), whereas, in children, the opposite increase in episodic memory recall

from Pre-sleep to the Sleep condition failed to reach significance ($t_{(34.16)} = -1.19$, $p = .24$).

Episodic memory components

We examined to what extent the enhancing effects of sleep and the age-dependent

differences in the dynamics (from short 1-hour Pre-sleep to longer-term recall after Sleep) on

episodic memory recall also emerged for the "What", "What–Where", and "What–When"

sub-components of episodic memory (Figure 2). Explicit recall of the subcomponents of

episodic memory was above chance in all six experimental groups (all $p < .05$), except for the

"What-When"-memory performance of the adult Wake group. "What" memory (i.e., the

percentage of familiar faces correctly judged as belonging to one of the two episodes) was

not significantly influenced by sleep compared to wakefulness ($p > .065$, for all analyses), but,

like episodic memory recall, showed superior recall rates at the 1-hour Pre-sleep test in adults,

and a stronger forgetting from Pre-sleep recall to recall testing after Sleep, in comparison

with children ($F_{(1, 78)} = 7.17$, $p = .009$, for Age × Pre-sleep vs. sleep, see Figure 2 for *post*

hoc tests). "What–Where" memory (i.e., the proportion of faces, for which the location was

correctly remembered), was generally better in adults than children ($F_{(1,75)} = 7.36$, $p = .008$,

for main effect Age group) and was enhanced by sleep in both age groups ($F_{(1,75)} = 11.95$, p

$< .001$, for main effect Sleep vs. wake), with the size of this enhancement being comparable

between age groups ($p = .92$, for Age group × Sleep vs. wake, recall rates – children Sleep:

55.57 ± 4.66%, Wake: 38.71 ± 4.64%, $t_{(38)} = -2.56$, $p = .01$, $d = 0.81$, adults Sleep: 69.39 ±

3.98%, Wake: 51.62 ± 7.28%, $t_{(37)} = -2.34$, $p = .02$, $d = 0.75$). Furthermore, "What–Where"

memory was markedly superior in adults compared to children at the Pre-sleep testing ($p <$ .001, Mann-Whitney U test), but tended to show larger differences between the Pre-sleep testing to the testing after Sleep in adults than in children ($F_{(1, 78)} = 3.88$, $p = .052$, for Age group $\times$ Pre-sleep vs. sleep), although the interaction term was only marginally significant. For the "What–When" memory component (i.e., the proportion of recalled faces for which the episode was correctly recalled), neither the enhancing effect of sleep ($p > .25$, for the respective analyses), nor the stronger forgetting from Pre-sleep to testing after Sleep in adults compared to children, reached significance ($p = .16$, for Age group $\times$ Pre-sleep vs. sleep).

We also examined capabilities to correctly recognize a face as familiar, which also included those eight faces presented only during the familiarization phase but not during one of the experimental episodes. The overall ANOVA revealed a significant effect of age group only ($F_{(1,110)} = 10.66$, $p = .002$) indicating better performance in adults than children in general. This age effect appeared to be driven by the faces only presented during the face familiarization phase ($F_{(1,110)} = 14.86$, $p < .001$) but did not occur for faces presented again in the episodes ($p > .72$) indicating that face recognition was comparable across age groups with respect to episodic encoding.

Sleep parameters and associations with memory performance

Table 1 summarizes the sleep architecture for the two sleep groups, as well as results from statistical comparisons between the age group. As expected, total sleep time was longer in the children than in the adults. After sleep onset, children reached slow-wave sleep (SWS) earlier and REM sleep later than adults did. In general, children spent less time in light sleep

stages (stage 1 and 2) and spent less time in wake after sleep onset (WASO). Sleep in

children and adults contained comparable proportions of REM and NonREM sleep (stage 2

plus SWS), but proportions of SWS were considerably greater in children than in adults (see

also Figure 1A). Table 1 also includes average power in selected EEG frequency bands,

considered relevant for sleep-dependent memory consolidation (see Figure S1, for respective

spectra), as well as the essential parameters for EEG slow oscillations and spindles in the two

groups. Importantly, the increased proportion of SWS in children was associated with a

greater number and density of slow oscillations ($p < .01$) and a greater density of fast

(centro-parietal) spindles ($p < .01$) and slow (frontal) spindles ($p < .05$) than in adults (p

$< .05$, see Table S1 for more detailed analyses).

We explored associations between episodic memory performance ("What–Where–

When" recall) and sleep parameters (as listed in Table 1) applying correlation analyses

separately on the two age groups. None of these sleep parameters were consistently

correlated with the episodic memory score.

Vigilance and sleepiness

We measured vigilance (using the PVT) and subjective sleepiness (with the SSS),

before encoding of each episode as well as before recall testing, to control for possible

confounding effects of changes in executive function. As expected, the children showed

generally slower reactions times on the PVT than adults (425.39 ± 13.87 ms vs. $306.08 \pm$

5.18 ms, $p < .001$). On the other hand, subjective sleepiness was lower in the children than in

the adults (2.22 ± 0.09 vs. 2.86 ± 0.09, $p < .001$). Because of these general differences

between the age groups, the subsequent analyses concentrated on differences between the

retention conditions, assessed separately for the age groups. In children, PVT performance

did not differ between Pre-sleep, Sleep, and Wake conditions, neither at encoding nor at

retrieval (all $p > .24$). Subjective sleepiness in the children was lower in the Pre-sleep group

than in the two other groups at encoding ($p < .007$ for both comparisons) but did not differ

between the retention groups at retrieval testing ($p > .19$).

The adult groups did not differ in PVT reaction times at encoding ($p > .07$ for

One-Way ANOVA). However, in the retrieval phase, the reaction time of the Wake group was

shorter than in the two other groups ($p < .043$ for both comparisons). The corresponding

pattern was obtained for subjective sleepiness which did not differ between adult groups at

encoding ($p > .2$ for both comparisons), but revealed that the Wake group felt less sleepy than

the two other groups at retrieval testing ($p < .012$, for both comparisons).

We additionally calculated correlations between vigilance measures at retrieval

testing and episodic memory recall for the retention conditions in both age groups separately.

These correlations neither reached significance for PVT reaction times (all absolute $r < 0.26$,

$p > .29$) nor for rated sleepiness (all absolute $r < 0.37$, $p > .13$) thus excluding that episodic

recall performance was substantially confounded by non-specific change in vigilance across

the retention conditions.

Discussion

Our results provide evidence for differential temporal dynamics of episodic memory

consolidation in children and adults. After a short 1-hour retention interval, the 8–12 years

old children showed distinctly lower recall than adult controls. By contrast, after a long

~10.5-hour retention interval, children's episodic memory recall was comparable with that in

adults. This was due to substantial further forgetting in adults, i.e., a decrease in recall

observed after the long retention intervals when compared with recall after the short interval,

whereas no such further forgetting was observed in the children. Sleep in the long 10.5-hour

interval, compared with wakefulness, enhanced episodic memory in both age groups to a

similar extent, although children spent distinctly more time in slow-wave sleep (SWS).

Increased density of slow oscillations and spindles also indicated that SWS was deeper in

children than in adults.

The benefit of intervening sleep on episodic memory recall confirms previous

findings in adult humans and rodents (Oyanedel et al., 2014; Racsmány, Conway, & Demeter,

2010; van der Helm, Gujar, Nishida, & Walker, 2011; Weber et al., 2014). Like those studies,

the current study also showed an enhancing effect of sleep on the "where" component of

episodic memory that was irrespective of age. Effects on the "when" component appear to be

consistently less robust (e.g., Oyanedel et al., 2014) for both age groups, which might reflect

that the task paradigm is less sensitive to this component. There is also an ongoing

conceptual debate to what extent hippocampal representations directly encode temporal

aspects of an episode (Easton, Webster, & Eacott, 2012). Item or "what" memory did not

benefit from sleep, which agrees with the view that sleep preferentially strengthens

hippocampus-dependent memory (Diekelmann & Born, 2010; Inostroza & Born, 2013;

Mumby, Tremblay, Lecluse, & Lehmann, 2005). Thus, the present study is the first to

demonstrate a beneficial effect of sleep on hippocampus-dependent genuinely episodic memory in preadolescent children.

Notably, the magnitude of the sleep-induced enhancement in episodic memory recall was similar in children and adults. This finding was unexpected, because the children showed more and deeper SWS, i.e., the sleep stage thought to be most relevant for the consolidation of hippocampus-dependent memory (Marshall & Born, 2007). Indeed, in several previous studies sleep-induced benefits on declarative types of memory in children appeared to be of a roughly comparable size to what is observed in adults (e.g., Henderson, Weighall, Brown, & Gaskell, 2012; Wilhelm, Diekelmann, & Born, 2008). However, those studies used tasks like word-pair learning, which probably do not reflect genuine episodic memory formation (Pause, Jungbluth, Adolph, Pietrowsky, & Dere, 2010). By contrast, children showed a distinctly superior benefit from sleep on a task requiring the abstraction of explicit knowledge from implicitly learned materials (Wilhelm et al., 2013). In that study, children at the age of 8–11 years were trained on a classical serial reaction time task under implicit conditions (i.e., not knowing about the underlying sequence in the task). Compared with wakefulness, post-training sleep benefitted explicit sequence knowledge, and this benefit was distinctly larger in the children than adults. Moreover, correlational analyses suggested this greater benefit to be linked to an enhanced slow wave activity in those children. Thus, SWS in children seemed to exert a stronger reorganizing effect on the task representations, thus allowing a greater gain of explicit knowledge. The different outcomes – similar memory enhancement but stronger memory reorganization after sleep in children, compared with

adults – might be reconciled based on concepts proposing that neural reactivations of hippocampal representations exert a twofold action on episodic representations: on the one hand they strengthen the hippocampal representation itself and, on the other hand, they support the gradual redistribution and reorganization of the representation (Inostroza & Born, 2013). In the present study testing episodic memory recall, we examined effects of sleep on the representation itself rather than its reorganizing influence. Thus, in the proposed conceptual framework, the present data in combination with previous findings of a superior reorganizing effect of sleep in children on memory representations (Ashworth, Hill, Karmiloff-Smith, & Dimitriou, 2014; Urbain et al., 2016; Wilhelm et al., 2013), suggest that the effect of sleep (and associated neural reactivations) on hippocampal episodic memory representation itself is comparable in middle childhood and adulthood but might be superior specifically in terms of the redistribution and reorganization of the representations.

This conclusion is also consistent with the present observation of episodic memory recall being unrelated to sleep slow oscillatory and spindle activity, because these rhythms might be more closely linked to the transfer of reactivated hippocampal memory information (Bergmann, Mölle, Diedrichs, Born, & Siebner, 2012; Staresina et al., 2015), rather than to the strengthening of the episodic memory itself. In this view, the strengthening of the episodic memory representation itself is linked to neural reactivations, associated with so-called hippocampal sharp wave-ripple complexes, a cognitive biomarker for the replay of episodic events which are indicated by fast synchronous network oscillations (ripples) in hippocampal regions which output to the neocortex. They occur mainly during quiet

wakefulness and SWS and are thought to assist in the transfer of hippocampal memory representations and redistribution to cortical circuits for the support of memory consolidation. They occur in synchrony with both the up-state of the slow oscillation and the troughs of the spindles but, their numbers remain unchanged by top-down influences of slow oscillations or spindles (Bendor & Wilson, 2012; Buszaki 2015).

Still, it could be argued that differences in pre-sleep memory strength between children and adults confounded the effects of subsequent sleep. At retrieval testing after the 1-hour short retention interval before sleep, recall of episodic memory was higher in the adults than in children suggesting a weaker memory strength in children at the time of falling asleep. Indeed, on a procedural motor sequence learning task, children who in general showed distinctly lower performance levels than adults, improved in the motor skill across sleep only after they underwent a pre-training to enhance their performance level (Wilhelm, Metzkow-Mészáros, Knapp, & Born, 2012). The greatest sleep-dependent benefits were revealed at an intermediate pre-sleep performance level which is consistent with findings in adults displaying greater sleep-dependent gains for weaker than stronger procedural memory traces (Kuriyama, Stickgold, & Walker, 2004). However, it is unclear to what extent these observations can be generalized to hippocampus-dependent types of episodic and declarative memory. There are hints from studies in adults that consolidation during sleep favors weakly over strongly encoded memories (Diekelmann, Born, & Wagner, 2010; Drosopoulos, Schulze, Fischer, & Born, 2007). Obviously, these observations would not explain that the children of the present study, showing a weaker pre-sleep strength of episodic memory than the adults,

did not display the expected superior sleep-induced enhancement of episodic memory.

The difference in recall after the short 1-hour pre-sleep interval between the age groups was indeed profound, with recall rates in the adults reaching average levels more than twice as high as those in the children. Whereas previous studies did not provide any evidence that episodic memory in children is very rapidly forgotten within 1 hour, they have consistently shown a less effective encoding of such memories in children (Guillery-Girard et al., 2013; Picard et al., 2012; Rollins & Riggins, 2013; Yim et al., 2013). Thus, the distinctly reduced episodic memory recall in children following the short 1-hour retention interval most likely reflects diminished capabilities to encode episodes in a coherent fashion in space and time, although in the present experiments we did not directly examine encoding by an immediate recall test. Moreover, the differences in tasks and reported memory components to previous studies cautions us to compare the differing concepts of temporal memory (e.g. like relational item-sequence memory in Guillery-Girard et al., 2013 and Lee et al. 2016). Diminished episodic memory encoding in children has been attributed to the protracted maturation of underlying brain structures, in particular of the prefrontal cortex but also of the hippocampus (Ghetti & Bunge, 2012; Gogtay et al., 2006; Seress & Abraham, 2008). This is consistent with maturation of the "where" memory processes up into adulthood (Guillery-Girard et al., 2013), which showed the strongest age-related differences in the current study as well. Furthermore, the most robust sleep benefit for the "where" component for children in particular suggests that these maturational changes might happen most effectively during sleep in this age group. This is consistent with less indication for

developmental trajectories for "when" or "what" memory in our sample (e.g. like in Picard et al., 2012) showing less or no sleep benefit due to slower development or processes independent of sleep.

Because of the immaturity of mainly the prefrontal cortex, children display generally diminished executive control functions (Hsu & Jaeggi, 2014; Picard, Reffuveille, Eustache, & Piolino, 2009). Accordingly, compared to the adults, the children of this study showed diminished performance on the Psychomotor Vigilance Task both during encoding and retrieval with this task specifically assessing the vigilance component of executive control, i.e., the capability to maintain attention over time. Such non-specific reduction in executive control and vigilance might also have lowered memory performance in the children, although within the group of children, episodic memory recall after the short or long retention interval did not significantly correlate with performance on the PVT. Also, self-reported sleepiness (SSS) could not explain the lowered performance (like the PVT it was not correlated with episodic memory recall), though this measure might be less sensitive (especially considering that it has not been specifically validated for use in children).

Importantly, however, even if diminished executive control lowered memory encoding and retrieval in general in the children, this could not explain the main finding of this study, i.e., that unlike adults showing strong forgetting of episodic memory from the short to the long retention interval, children did not show such decrease in recall but, maintained recall performance levels over the long interval. This finding also cannot be questioned based on the relatively small subgroup sample sizes (especially the adult awake

group) which might be considered another limitation of the study. It rather seems justified to conclude from this data that, independently of sleep or wakefulness, children showed more effective consolidation of episodic memory. The underlying mechanisms are unclear. Hippocampal ripples accompanying the reactivation of episodic representations might be involved as they occur both during SWS and quiet wakefulness (Buzsáki, 2015; Clemens et al., 2007). Although the occurrence of ripples does not appear to be increased during childhood, they might more effectively induce plastic synaptic changes (Buhl & Buzsáki, 2005).

In sum, we used a genuine episodic memory task, which enabled us to directly compare retention of these memories after short (1 hour) and long (10.5 hour) intervals between 8-12 years old children and adults. Diminished retention at the 1-hour recall suggests that children had already encoded the episodes less effectively than the adults, although this conclusion has to be scrutinized in further studies examining encoding using immediate recall tests. On the other hand, children showed, in contrast to adults, no significant signs of further forgetting this information across the 10.5-hour interval. The mechanisms underlying enhanced consolidation in children are independent of sleep.

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Tables

Table 1

Sleep parameters and correlations with episodic memory recall

| Sleep parameter			Correlations with memory recall [*r*]	
Stages [min]	**Children (*n* = 18)**	**Adults (*n* = 24)**	**Children (*n* = 17)**	**Adults (*n* = 24)**
TST	553.19 ± 3.45 ***	468.27 ± 2.80	-.20	-.10
Sleep onset	15.80 ± 3.97	15.66 ± 2.46	-.07	.04
SWS latency	8.78 ± 0.76 ***	15.98 ± 2.35	-.03	-.31
REM latency	117.61 ± 10.35 **	88.04 ± 6.57	-.07	.12
WASO	1.39 ± 0.88 ***	13.60 ± 5.09	.08	.22
Stage 1	11.47 ± 1.01 **	20.00 ± 2.62	.20	-.12
Stage 2	120.03 ± 9.09 ***	228.56 ± 8.28	-.06	-.11
SWS	275.86 ± 10.81 ***	97.79 ± 7.28	-.12	.05
NonREM	395.89 ± 6.44 ***	326.35 ± 6.99	-.30	-.08
REM	138.67 ± 4.79 ***	104.17 ± 5.65	.17	-.10
Stages - % of TST				
WASO	0.25 ± 0.16 ***	2.96 ± 1.13	.08	.24
Stage 1	2.07 ± 0.18 **	4.29 ± 0.57	.22	-.10
Stage 2	21.66 ± 1.61 ***	48.73 ± 1.64	-.05	-.11
SWS	49.89 ± 1.97 ***	20.94 ± 1.59	-.09	.05
NonREM	71.56 ± 1.04	69.67 ± 1.41	-.25	-.07
REM	25.07 ± 0.86	22.19 ± 1.16	.21	-.10
SWA, 0.5–4 Hz, in SWS				
(Fz,Cz,Pz) [μV^2/Hz]	539.08 ± 46.19 ***	211.64 ± 17.21	-.11	-.04
Spindle activity, 9–15 Hz, in NonREM				
(Fz,Cz,Pz) [μV^2/Hz]	4.70 ± 0.68 ***	2.31 ± 0.22	-.05	-.06
Slow oscillations in NonREM (at Fz)				
Count	2616 ± 66 ***	1833 ± 76	-.47 *	.05
Density [1/min]	7.63 ± 0.15 ***	6.36 ± 0.21	-.25	.05
Amplitude [μV]	274.49 ± 11.93 ***	177.84 ± 7.94	-.03	.11
Slope [μV/s]	651.13 ± 34.08 ***	447.59 ± 23.79	.01	.05
Fast spindles in NonREM (at Cz)				
Count	1658 ± 52	1532 ± 58	-.44	.17
Density [1/min]	4.84 ± 0.13 *	5.30 ± 0.12	-.28	.28
Core frequency [Hz]	12.22 ± 0.14 ***	13.31 ± 0.12	-.03	.22
Slow spindles in SWS (at Fz)				
Count	1191 ± 52 ***	417 ± 48	-.06	.13
Density [1/min]	4.87 ± 0.18	4.60 ± 0.39	.11	.20
Core frequency [Hz]	11.21 ± 0.15	11.20 ± 0.16	-.10	.22

Means ± SEM for the sleep parameters are shown in the left columns for children compared with adults. The right columns show Pearson's correlation of sleep parameters with episodic memory recall ("What-Where-When"). Given are the total sleep time (TST), sleep onset (with reference to the time of lights off), latency for slow wave sleep (SWS) and rapid eye movement (REM) sleep (with reference to sleep onset) and time spent awake after sleep onset (WASO), sleep stage 1, sleep stage 2, SWS, NonREM (S2 + SWS) and REM in minutes and percentage of total sleep time. In addition, slow wave activity (SWA, 0.5–4 Hz) and spindle activity (9–15 Hz) both averaged across Fz, Cz, and Pz, are indicated. For identified slow oscillation and fast and slow spindles events, the absolute count, density (per minute), and average amplitude is given. Additionally, the average slope of the down-to-upstate transition is indicated for the slow oscillation, as well as the core frequency (cycles per second of identified events) for spindles. Slow oscillation and spindle parameters are given for the site of the typical maximum of these events (Fz and Cz, respectively). *** p < .001, ** p < .01, * p < .05

Figures
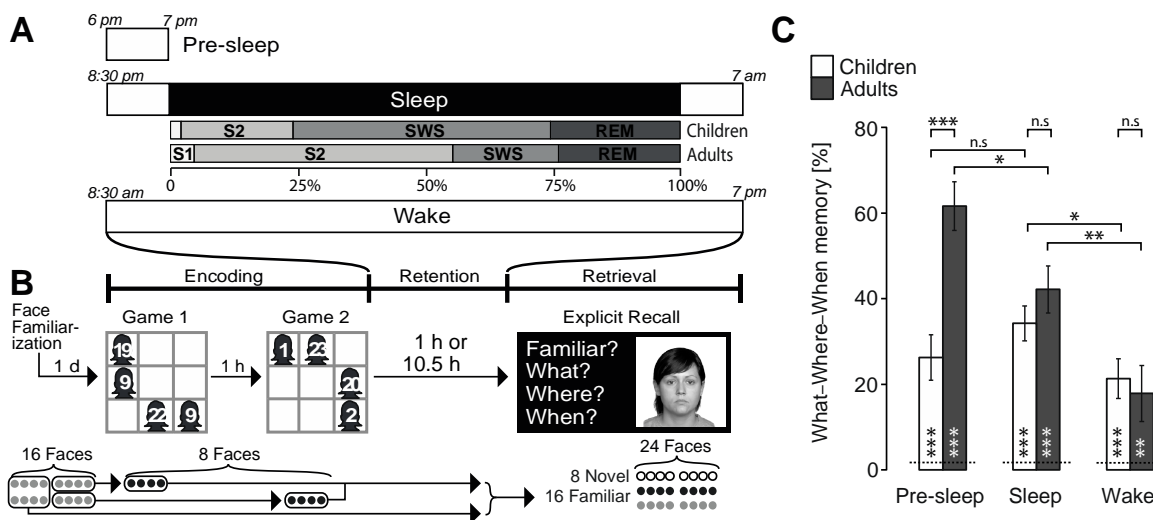
Figure 1. Experimental design, task procedures, and episodic memory recall. **(A)** Different

groups of children (8–12 years) and adults were tested in three Retention conditions, i.e., the

Pre-sleep, Sleep, and Wake condition. Each condition comprised an encoding, a retention,

and a retrieval phase, with only the retention phase differing between conditions: In the

Pre-sleep condition, the retention phase covered a short 1-hour interval in the early evening.

In the Sleep and Wake conditions the retention interval was longer (~10.5 hours) and covered

periods of nocturnal sleep or daytime wakefulness, respectively. Time of the end of encoding

(after the second episode was encoded) and the start of retrieval are marked exemplary for

children, but in fact were timed following the individual and group specific sleep habits (see

Methods). Grey bars under the Sleep retention condition indicate the average percentages of

total sleep time children and adults spent in the different sleep stages. Compared to adults,

children had higher percentages of slow wave sleep (SWS) and lower percentages of lighter

stage 1 and 2 sleep. Percentages of REM sleep were comparable for both age groups. **(B)** The

encoding phase of the episodic memory task comprised two episodes (Game 1, Game 2) separated by a 1-hour interval, each entailing the presentation of a specific configuration of four individual faces in a 3×3 grid of locations. In the retrieval phase, explicit recall of episodic memory was assessed by presenting 24 faces and asking whether (i) the face was new or familiar; (ii) if familiar, whether it occurred in one of the games or not (What), and (iii) if it was Game 1 or Game 2 (When). (iv) If the participant indicated that the face occurred in one of the Games, he or she should indicate at which location the face occurred (Where, shown together with the empty grid) (see Methods for details). The bottom part illustrates the faces used in the different experimental phases: the face familiarization phase took place on the day before the experiment proper, subjects were familiarized with 16 faces (gray circles), of which 8 faces were used in the encoding phase of the episodic memory task (4 in each game). At recall testing 24 faces were presented, i.e., the 16 familiarized faces (8 from the games - black circles, 8 not in the games but presented in the familiarization phase - gray circles), and 8 entirely novel faces (empty circles). These latter faces allowed to discriminate between "face recognition" (novel vs. familiar) and "What" memory (in the episodes vs. not in the episodes). (**C**) Mean ($\pm$ SEM) episodic memory ("What–Where–When") recall in children (empty bars) and adults (filled bars) for the different retention conditions (children Pre-Sleep, $n = 19$; Sleep, $n = 20$; Wake, $n = 20$; adults Pre-Sleep, $n = 18$; Sleep, $n = 25$; Wake, $n = 14$). $* p < .05$, $** p < .01$, $*** p < .001$, for post hoc pairwise comparisons. Asterisks in the bars indicate significance for above chance level performance (dotted line).
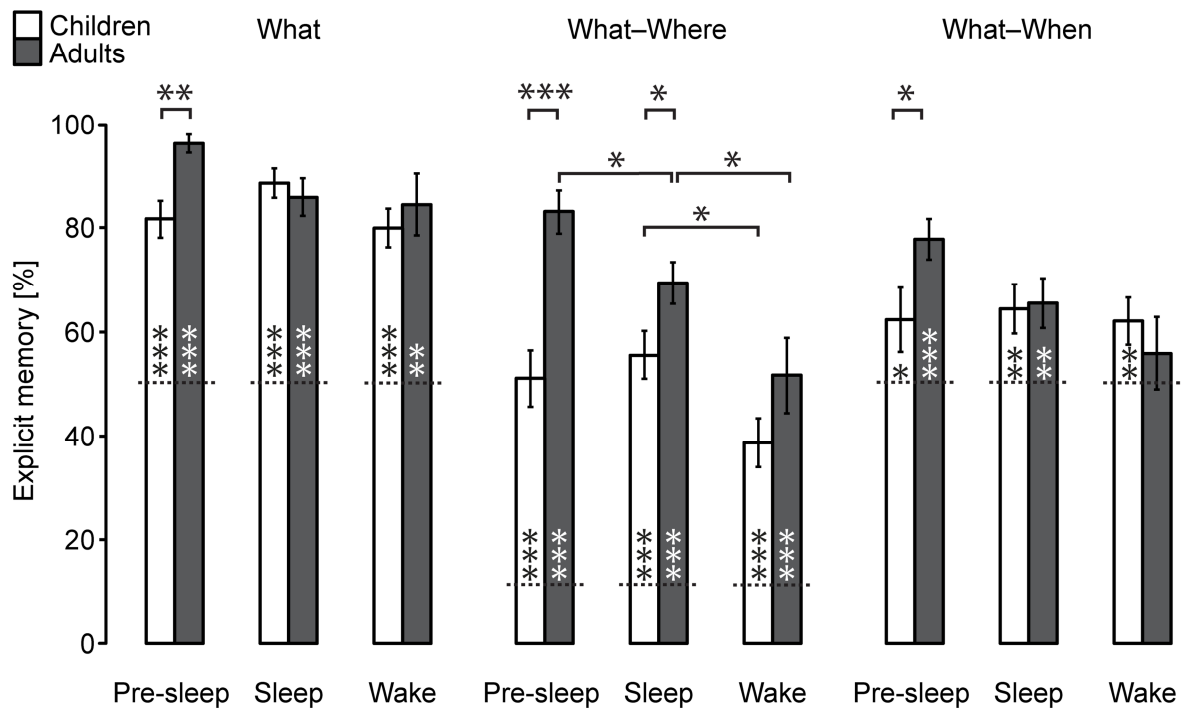
Figure 2. Mean (± SEM) recall for sub-components of episodic memory ("What", "What–Where", "What–When") in children (empty bars) and adults (filled bars) for the different retention conditions (children Pre-Sleep, $n = 19$; Sleep, $n = 20$; Wake, $n = 20$; adults Pre-Sleep, $n = 18$; Sleep, $n = 25$; Wake, $n = 14$). $* p < .05$, $** p < .01$, $*** p < .001$, for post hoc pairwise comparisons. Asterisks in the bars indicate significances for above chance level performance (dotted line).

Supporting Information

Additional Sleep EEG Analyses

Analysis was performed using the SpiSOP tool (http://www.spisop.org) based on MATLAB 2013b (Mathworks, Natick, USA) and FieldTrip (Oostenveld, Fries, Maris, & Schoffelen, 2011, http://www.ru.nl/neuroimaging/fieldtrip).

Power spectral analyses of NonREM sleep. Power spectra were calculated on consecutive artifact-free 5-s intervals of NonREM sleep, which overlapped in time by 4 s. Each interval was tapered by a single Hanning window before applying Fast Fourier Transformation that resulted in interval power spectra with a frequency resolution of 0.2 Hz. Power spectra were then averaged across all blocks (Welch's method) and normalized by the effective noise bandwidth to obtain power spectral density estimates for the whole data. Mean power density in the following frequency bands was determined: slow-wave activity (0.5–4 Hz), theta (4–8 Hz), spindles (9–15 Hz), slow spindles (9–12 Hz) and fast spindles (12–15 Hz), and log transformed (decibel) prior to statistical testing.

Slow oscillations. Identification of slow oscillations was based on a previously published algorithm (Mölle, Marshall, Gais, & Born, 2002). For each EEG channel, the signal during NonREM epochs was filtered between 0.5 and 3.5 Hz (-3 dB roll-off) using a digital FIR filter (Butterworth, order of 4). Then all time intervals with consecutive positive-to-negative zero crossings were marked as putative slow oscillation if their durations corresponded to a frequency between 0.5 and 1.11 Hz (Ngo, Martinetz, Born, & Mölle, 2013). Putative slow oscillations were immediately excluded with an amplitude >1000 µV (as these were considered artifacts) or when both negative and positive half-wave amplitudes were smaller than -15 µV and +10 µV, respectively. A slow oscillation was then identified if its negative half-wave peak potential was lower than the mean negative half-wave peak of all putatively detected slow oscillations in the respective EEG channel, and also only if the

amplitude of the positive half-wave peak was larger than the mean positive half-wave amplitude of all other putatively detected slow oscillations within this channel. For each individual and channel, the number of slow oscillations, their density (per min NonREM sleep), mean amplitude, and slope (the ratio between absolute value of the negative half-wave peak and the time to the next zero crossing; Riedner et al., 2007) were calculated.

Spindles. For the detection of spindles, the EEG signal was filtered between 9 and 15 Hz (-3dB roll off). Then, using a sliding window with a size of 0.2 s the root mean square (RMS) was computed and the resulting signal was smoothed in the same window with a moving average. A spindle was detected when the smoothed RMS signal exceeded an individual amplitude threshold 1.75 times the standard deviation of the filtered signal in this channel at least once, and additionally, exceeded a lower threshold of 1.5 standard deviations for 0.5–3 s. The crossings of the lower threshold marked the beginning and end of each spindle. Spindle amplitude was defined by the voltage difference between the largest trough and the largest peak. Spindles were excluded with amplitudes higher than 200 µV. For a separate detection of slow and fast spindles, respective frequency peaks were visually identified in individual power spectra of all NonREM sleep epochs. According to their expected power maxima (Mölle, Bergmann, Marshall, & Born, 2011), slow spindle peaks were identified in frontal channels (F3, Fz, F4) and fast spindle peaks were identified in centro-parietal (C3, Cz, C4, P3, Pz, P4). In two children and two adults clear slow spindle peaks were not manifested and the mean of the age group was taken instead. For each EEG channel, the NonREM epochs were filtered with a band-pass of ± 1 Hz (-3 dB cutoff) around the individual fast or slow spindle frequency peaks, respectively. The further detection procedure followed the same spindle detection algorithm described above. For each subject and channel absolute spindle counts, spindle density (per min NonREM and SWS for fast and

slow spindles, respectively), mean amplitude, average oscillatory frequency and duration

were calculated.

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Table S1

Properties of Slow Oscillations and Spindles in Children and Adults

Events	Children (*n* = 18)			Adults (*n* = 24)			*p*-value
Slow Oscillations							
Count Fz	2616	±	66	1833	±	76	<.001
Count Cz	2489	±	69	1644	±	69	<.001
Count Pz	2169	±	58	1618	±	80	<.001
Density [1/min] Fz	7.63	±	0.15	6.36	±	0.21	<.001
Density [1/min] Cz	7.25	±	0.11	5.71	±	0.20	<.001
Density [1/min] Pz	6.33	±	0.13	5.61	±	0.23	.011
Amplitude [µV] Fz	274.49	±	11.93	177.84	±	7.94	<.001
Amplitude [µV] Cz	282.69	±	11.99	161.60	±	6.72	<.001
Amplitude [µV] Pz	215.37	±	8.57	143.28	±	5.95	<.001
Frequency [Hz] Fz	0.84	±	0.00	0.83	±	0.01	.028
Frequency [Hz] Cz	0.84	±	0.00	0.82	±	0.01	.062
Frequency [Hz] Pz	0.85	±	0.00	0.81	±	0.01	<.001
Slope [µV/s] Fz	651.13	±	34.08	447.59	±	23.79	<.001
Slope [µV/s] Cz	650.80	±	29.63	376.04	±	17.88	<.001
Slope [µV/s] Pz	519.35	±	22.20	306.57	±	14.03	<.001
Spindles (9–15 Hz)							
Count Fz	1427	±	44	1146	±	47	<.001
Count Cz	1337	±	49	1144	±	46	.007
Count Pz	1145	±	56	1215	±	52	.36
Density [1/min] Fz	4.17	±	0.11	3.97	±	0.13	.26
Density [1/min] Cz	3.9	±	0.11	3.96	±	0.12	.69
Density [1/min] Pz	3.33	±	0.15	4.21	±	0.14	<.001
Amplitude [µV] Fz	71.17	±	3.58	47.82	±	2.26	<.001
Amplitude [µV] Cz	61.2	±	2.91	48.36	±	2.05	.001
Amplitude [µV] Pz	43.66	±	2.56	43.91	±	2	.94
Core frequency [Hz] Fz	11.44	±	0.14	11.54	±	0.16	.66
Core frequency [Hz] Cz	12.01	±	0.14	12.29	±	0.18	.22
Core frequency [Hz] Pz	11.88	±	0.15	12.51	±	0.19	.01
Duration [ms] Fz	897	±	13	821	±	8	<.001
Duration [ms] Cz	907	±	16	818	±	9	<.001
Duration [ms] Pz	920	±	13	854	±	11	<.001
Fast spindles							
Power peak [Hz]	12.26	±	0.12	13.40	±	0.12	<.001
Count Fz	1680	±	47	1402	±	56	<.001
Count Cz	1658	±	52	1532	±	58	.10
Count Pz	1532	±	51	1629	±	57	.21
Density [1/min] Fz	4.92	±	0.13	4.84	±	0.13	.69

Density [1/min] Cz	4.84	±	0.13	5.30	±	0.12	.011
Density [1/min] Pz	4.46	±	0.12	5.65	±	0.13	<.001
Amplitude [µV] Fz	50.90	±	2.90	28.00	±	1.23	<.001
Amplitude [µV] Cz	44.93	±	2.52	32.67	±	1.63	<.001
Amplitude [µV] Pz	31.78	±	2.24	30.90	±	1.64	.75
Core frequency [Hz] Fz	11.91	±	0.13	13.02	±	0.12	<.001
Core frequency [Hz] Cz	12.22	±	0.14	13.31	±	0.12	<.001
Core frequency [Hz] Pz	12.16	±	0.14	13.35	±	0.12	<.001
Duration [ms] Fz	936	±	16	812	±	10	<.001
Duration [ms] Cz	980	±	20	841	±	14	<.001
Duration [ms] Pz	1013	±	16	888	±	15	<.001

Slow Spindles

Power peak [Hz]	11.06	±	0.13	11.27	±	0.14	.27
Count Fz	1585	±	51	1200	±	61	<.001
Count Cz	1364	±	55	1033	±	50	<.001
Count Pz	1191	±	63	909	±	48	.001
Density [1/min] Fz	4.63	±	0.14	4.15	±	0.18	.040
Density [1/min] Cz	3.97	±	0.14	3.58	±	0.14	.053
Density [1/min] Pz	3.46	±	0.17	3.14	±	0.13	.12
Amplitude [µV] Fz	53.20	±	2.66	31.37	±	1.36	<.001
Amplitude [µV] Cz	42.30	±	2.34	28.06	±	1.06	<.001
Amplitude [µV] Pz	30.46	±	1.90	23.99	±	1.01	.005
Core frequency [Hz] Fz	11.21	±	0.15	11.20	±	0.16	.95
Core frequency [Hz] Cz	11.47	±	0.17	11.30	±	0.18	.50
Core frequency [Hz] Pz	11.49	±	0.17	11.36	±	0.21	.63
Duration [ms] Fz	924	±	13	828	±	8	<.001
Duration [ms] Cz	942	±	19	814	±	11	<.001
Duration [ms] Pz	960	±	24	847	±	19	<.001

Means ± SEM parameters (at Fz, Cz, and Pz location) of slow oscillation and spindle events identified during NonREM sleep in the Sleep groups of children and adults. For slow oscillation events the count, density, average amplitude, average frequency (as derived from the period length), and the slope of the down-to-upstate transition is indicated. For spindles (9-15 Hz) and subclasses of fast and slow spindles (the latter identified based on the frequency of the individual power peak in the spectrum) the frequency of the power peak, the count, density, average amplitude, core frequency (defined by the average number of cycles/s), and the duration is indicated. The right column indicates significance level for

direct comparisons between age groups. The most important results were: compared to adults, children displayed higher counts, density, average amplitude, and greater slopes of slow oscillations. With regard to spindles, children showed higher counts and amplitude of fast spindles at Fz, but increased fast spindle density at Pz compared to adults. Fast spindle duration was generally longer and core frequency was generally slower than in adults. Slow spindle count and amplitude were higher in the children, in particular at Fz, and slow spindle duration was generally longer than in adults.
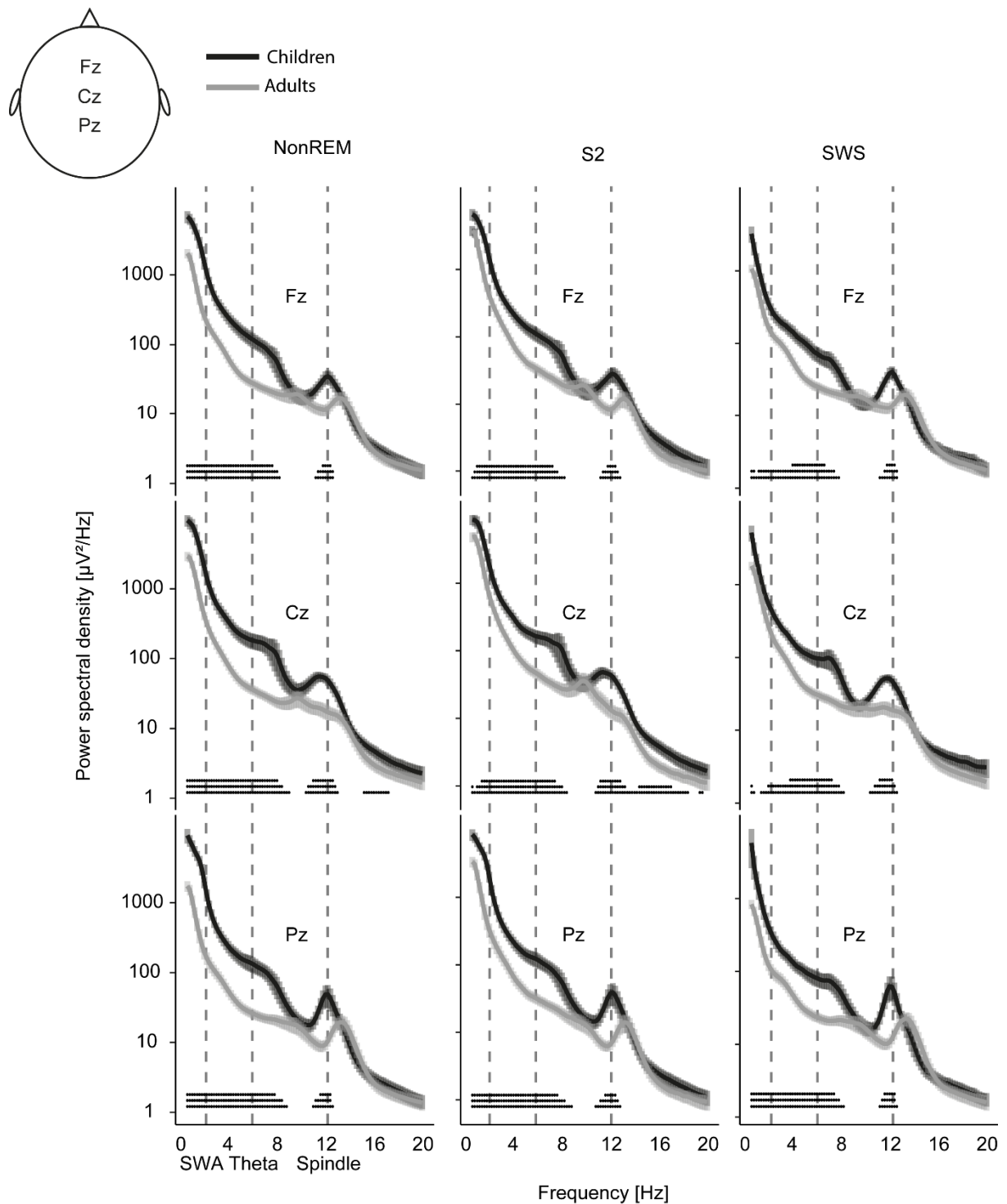
Figure S1. Mean ± SEM power density in children (black lines) and adults (grey) in NonREM sleep, as well as separately for slow wave sleep (SWS) and stage 2 (S2) sleep at Fz, Cz and Pz electrode sites (resolution 0.2 Hz). Spectra were normalized by multiplying with the mean of the total power density between 20 and 30 Hz. Stacked dots underneath the

spectra indicate significant differences between children and adults for each frequency bin

(one dot $p < .05$, two dots $p < .01$, three dots $p < .001$. Note, that power below 8 Hz and

around 12 Hz was generally increased in the children.

Study III:

Comparison for sleep's influence on semantic and episodic memory consolidation in children and adults

Manuscript: Effects of sleep on word-pair memory in children – separating item and source memory aspects.

Wang, JY., Weber, FD., Zinke, K., Noack, H., & Born, J.

Effects of sleep on word-pair memory in children – separating item and source memory aspects

Jing-Yi Wang [1,2], Frederik D. Weber [1], Katharina Zinke[1], Hannes Noack1[1], Jan Born[1,3]

[1]Institute of Medical Psychology and Behavioral Neurobiology, University of Tübingen, Tübingen, Germany

[2]Graduate School of Neural and Behavioral Sciences, University of Tübingen, Tübingen, Germany

[3]Werner Reichardt Center for Integrative Neuroscience, University of Tübingen, Tübingen, Germany

Author Note

Correspondence should be addressed to Jan Born, Department of Medical Psychology and Behavioral Neurobiology, University of Tübingen, Otfried-Müller-Str. 25, 72076 Tübingen, Germany. Email: jan.born@uni-tuebingen.de. Tel: +49(0)7071-29-88924. Fax: +49(0)7071-29-25016

Highlights

Sleep benefits word paired-associate learning in 8 to 12-year-old children.

Sleep does not specifically enhance episodic binding of word-pair to specific source.

Compared with adults, children mainly store word-pairs as items unbound to a source.

Abstract

Word paired-associate learning is a well-established task to demonstrate post-learning consolidation during sleep in adults as well as children. Sleep has been proposed to benefit newly encoded memory mainly by an impact on its episodic features, i.e., a memory for an event (item) bound into spatiotemporal context (source). In order to examine to what extent the enhancing effect of sleep on word-pair memory in children originates from an effect on the episodic representation of the task, we tested children (8-12 yrs., $n = 61$) on a modified paired-associate task where two lists of word-pairs were each studied once 1-hour apart. Retrieval testing comprised cued recall of the target word (considered item memory) and recall of the word-pair list (source memory), and took place either after 1 hour (short retention interval) or after 11 hours, with this long retention interval covering either nocturnal sleep or daytime wakefulness. Compared with the wake interval, sleep enhanced recall of both word-pairs and the lists per se, while the combined recall of word-pairs and the associated list remained unaffected. An additional comparison with adult controls ($n = 37$) suggested that item-source bound memory (combined recall of word-pair and list) is generally diminished in children. Our results argue against the view that the sleep-induced enhancement in word-pair memory in children is a consequence of sleep specifically enhancing the episodic task representation. On the contrary, sleep in children might have stronger unbinding effects on episodic representations, which are less developed than in adults.

Keywords: memory consolidation, electroencephalography, declarative memory, episodic memory, child development, sleep

Sleep facilitates memory consolidation with ample evidence, especially for declarative memories (Rasch & Born, 2013). Many of these studies have employed the declarative word paired-associate learning task. In this task, subjects study a list of associated word-pairs and cued recall is tested after a retention interval of specific length by presenting the first words of the pairs. Sleep compared to wakefulness after learning robustly enhances memory for the studied pairs, in adults (Payne et al., 2012; Plihal & Born, 1997) and children (Potkin & Bunney, 2012; Wilhelm, Diekelmann, & Born, 2008).

The beneficial effect of sleep on declarative memory consolidation has been assumed to rely on a process of system consolidation involving neural reactivations that primarily affect the episodic features of the encoded task representations residing in hippocampal networks (Diekelmann & Born, 2010; Inostroza & Born, 2013). Specifically, the hippocampus is thought to encode an episode by binding an event (item) into its spatiotemporal context (source). Thus, memory for episodic features, like information about when and where an event occurred crucially relies on hippocampus (Devito & Eichenbaum, 2011; Lehn et al., 2009), and memory for such contextual information seems to be supported by sleep (Drosopoulos, Windau, Wagner, & Born, 2007; van der Helm, Gujar, Nishida, & Walker, 2011). Moreover, sleep also appears to support the binding of item memory into source memory which is characteristic for episodic memory (Inostroza, Binder, & Born, 2013; Oyanedel et al., 2014; Weber, Wang, Born, & Inostroza, 2014), although other studies show the opposite, i.e., a 'de-contextualizing' effect of post-encoding sleep enhancing the unbinding of episodic memory such that the memory for items becomes less dependent on the spatiotemporal source in which it was learned (Cairney, Durrant, Musgrove, & Lewis, 2011; Deliens & Peigneux, 2014; Sweegers & Talamini, 2014).

Children show robust abilities to form memories for events (items) early in development

(Mullaly & Maguire, 2014). However, memory for source information, like the spatial and temporal context an event has occurred in, shows a protracted trajectory of development throughout the first decade of life and even beyond (Bauer & Lukowski, 2010; Picard, Cousin, Guillery-Girard, Eustache, & Piolino, 2012) with a distinct developmental trajectory for binding item and source (Riggins, 2014). This slow development appears to be partly due to the protracted maturation of the brain structures involved in episodic memory formation (Ghetti & Bunge, 2012; Gogtay et al., 2006). However, children's sleep is also longer and deeper, with higher proportions of slow-wave sleep (SWS) containing more intense slow-wave activity, reaching a maximum in preadolescence (Ohayon, Carskadon, Guilleminault, & Vitiello, 2004; Huber & Born, 2014; Wilhelm et al., 2014). Because processes during SWS such as slow wave activity and associated spindle activity, are implicated in the consolidation of declarative memory (e.g., Marshall, Helgadóttir, Mölle, & Born, 2006; Ngo, Martinetz, Born, & Mölle, 2013), children might be expected to display enhanced sleep-dependent memory consolidation, despite a less developed episodic memory system.

Against this backdrop, our study aimed to dissociate to what extent the enhancing effect of sleep on word-pair memory in 8-12-year old children might originate from strengthening the underlying episodic task representation. We relied on word paired-associate learning because it has consistently reflected the memory-enhancing effects of sleep in previous studies. But, the task was modified – comprising two different word-pair lists to be studied 1 hour apart – to discriminate effects of sleep on item and source memory and on the binding of these two aspects. Enhanced episodic memory consolidation during sleep was expected to increase, in particular, the number of recalled word-pairs for which also the list (source) was correctly recalled.

Methods

Participants

Sixty-one healthy children (8-12 years) without any known neurological or psychiatric disorder were recruited from local schools. Two children had to be excluded because of missing data, and one for taking a nap during wake retention. Participants were assigned to three experimental groups with age and gender balanced (Pre-Sleep: 9.65 ± 0.27 years, $n = 20$, 9 males; Sleep group: 9.9 ± 0.24 years, $n = 21$, 10 males; Wake group: 10.1 ± 0.28 years, $n = 17$, 10 males). To compare the dynamics of memory retention between children and adults, an additional control sample of 37 adults (healthy German native speakers, 18-30 years) was recruited. They were either assigned to the Pre-sleep (23.13 ± 0.81 years, $n = 18$, 8 males) or the Sleep condition (23.32 ± 0.53 years, $n = 19$, 10 males), and basically followed the same procedure as the children participants. Participants were part of a larger study and performed another unrelated task, which will be reported elsewhere. The ethics committee of the local university approved this study.

Design and Procedures

The experimental procedure consisted of an encoding phase, a retention phase, and a retrieval phase. The retention interval was either short (1 hour) for children in the Pre-sleep group or long (11 hours) in the Sleep and Wake (10 hours for the Sleep adults, Figure 1A).

Participants of the sleep groups slept one night in the sleep lab with polysomnographic recording one day before the experiments. On the experiment night, children arrived at the lab about 3 h earlier than their usual bedtime. After the preparation for EEG, children encoded two lists of word-pairs between 6:00 pm and 8:00 pm with a 1-hour break that was filled with standardized lab activities (i.e. playing games with the experimenter). Children went to bed 30 minutes after the encoding phase completed and slept in the lab for about 9.5 h with polysomnographic recordings. The retrieval phase began 45 minutes after waking up and

consisted of the recall of word-pairs and their temporal context.

The encoding phase of the Wake group children took place between 7:00 am to 9:00 am at the experimental days. After the encoding phase, participants followed their normal daily routine outside the lab avoiding stressful mental and physical activities and were restrained from taking a nap, which was controlled with actigraphy (Actiwatch 2, Philips, Netherlands). With a retention interval of about 11 h, participants came back to the lab to complete the retrieval phase at around 7:00 pm.

Children in the Pre-sleep group came to the lab for the experimental evening about 3.5 h before their normal sleep time, and the encoding phase took place between 4:00 pm and 6:00 pm. The retrieval phase took place 1 h after the encoding phase was completed. Sleep recordings, EEG analyses, other control measures performed to exclude confounding influences of alterations in vigilance, as well as statistical analyses are described in detail the Supplementary material.
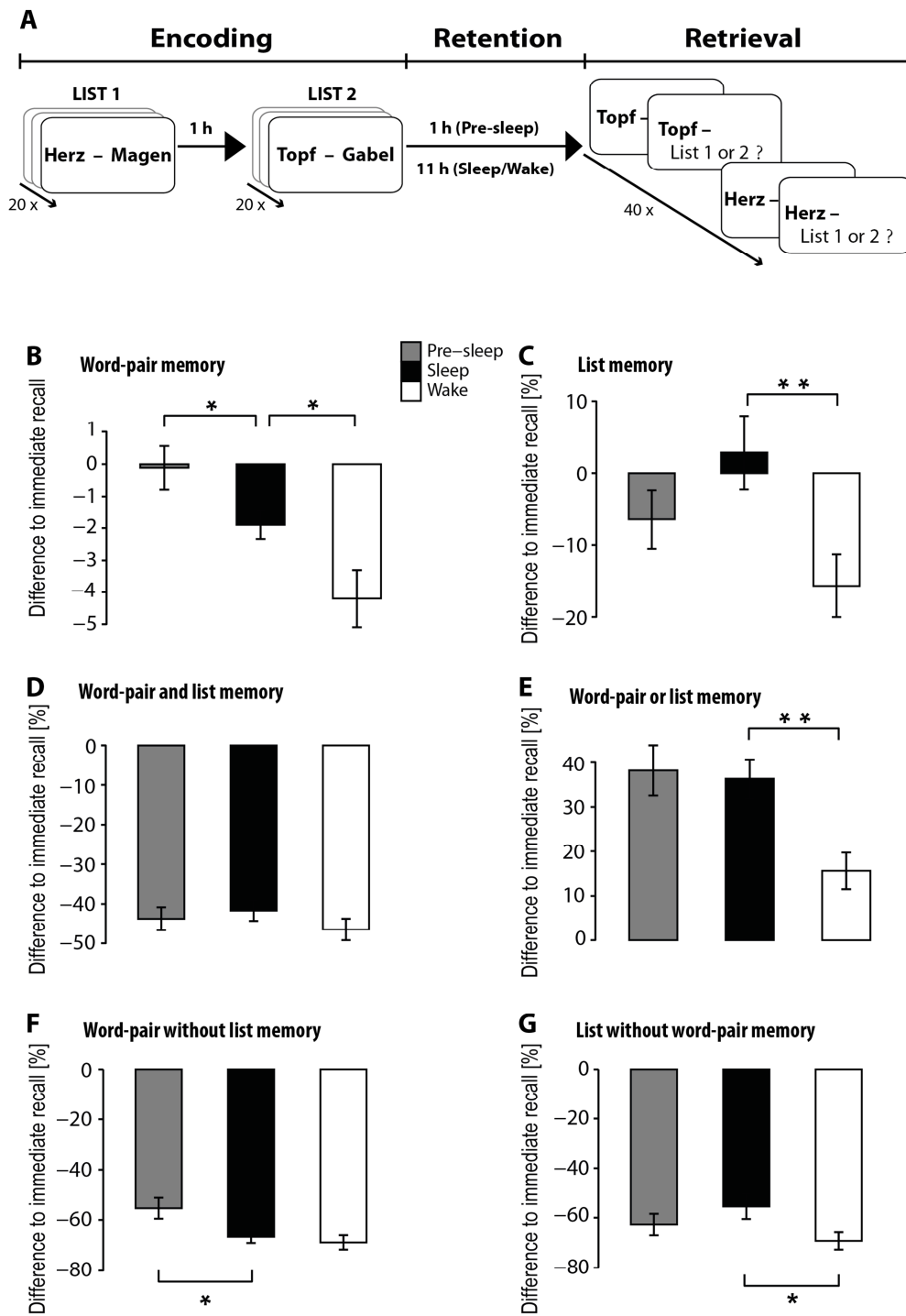
Word-pair learning

The paired-associate learning task comprised 40 (80 for adults) semantically related word-pairs and was adopted from a previous study (Wilhelm et al., 2008). These word-pairs were split randomly into two equally sized lists with the word-pairs in random order. During encoding, word-pairs were presented on the screen for 6 s with 1 s pause for children and 4 s with 1 s pause for adults, respectively. Participants were instructed to remember the word-pairs for a later recall (item memory). No instructions were given to remember the temporal context (list order), however, word-pair lists were introduced as "List 1" or "List 2" on the PC screen. Right after encoding of each list, children were shown one cue word of each word-pair on the PC screen and were asked to orally recall the corresponding target word without any feedback (immediate

recall). After the retention phase, delayed recall of word-pairs from both lists was tested in random order. Additionally, participants had to indicate in which of the two lists a specific word-pair (item) was presented originally (source memory).

The memory task was designed for one-time encoding, thus precluding the exclusion of poor performers right away. Therefore, poor performers with an average immediate word-pair recall below 40% were excluded from the analyses (Children: $n = 5$, adults: $n = 4$).

Results

Immediate recall of word-pairs in the children neither differed between the three retention groups (Pre-sleep, Sleep, Wake, $F_{(2, 50)} = 0.89$, $p > .4$) nor between List 1 and 2 ($F_{(1, 50)} = 0.85$, $p > .4$). Forgetting dynamics over the retention interval (measured as the difference in delayed recall of word-pair with respect to immediate recall) were, however, markedly different between retention groups ($F_{(2, 50)} = 8.96$, $p < .001$, one-way ANOVA). Across the short 1-hour retention interval (Pre-sleep), forgetting of word-pairs was virtually absent. Further forgetting across the longer 11-hour interval was reduced in the Sleep group compared to the Wake group ($t_{(29.72)} = 2.22$, $d = 0.73$, $p = .03$ for Pre-sleep vs. Sleep; $t_{(31)} = 3.71$ $d = 1.28$, $p < .001$ for Pre-sleep vs. Wake; $d = 0.82$, $t_{(20.71)} = 2.30$, $p = .03$ for Sleep vs. Wake, two-tailed t-tests, Figure 1B).

Figure 1. Experimental design, and memory dynamics in children. (**A**) The Encoding phase of the experiment consisted of learning two lists of word-pairs, each studied once one hour apart. The duration of the retention interval was either 1 hour (Pre-sleep condition) or 11 hours with the latter including either a night of sleep (Sleep condition)

or daytime wakefulness (Wake condition). In the retrieval phase cued recall was tested for each word-pair followed by a recall of the list (forced choice between List 1 or 2) in which the word-pair had appeared. (**B-G**) Children's mean (± SEM) cued recall performance for the Pre-sleep (gray bars), Sleep (black) and Wake (white) conditions for (**B**) correctly recalled word-pairs, (**C**) correctly recalled lists, (**D**) trials with both correctly recalled word-pairs and lists, (**E**) trials with correctly recalled word-pairs or lists, (**F**) trials with correctly recalled word-pairs but incorrect list recall, and (**G**) trials with correct list recall but incorrect word-pair recall. Recall is expressed as the difference from immediate recall of word-pairs during the encoding phase. * $p < .05$, ** $p < .01$, for pairwise comparisons between retention conditions.

The absolute number of words with correct list recall was comparable across conditions (Pre-sleep: 22.72 ± 0.97, Sleep: 24.00 ± 0.78, Wake: 22.07 ± 0.78, $F_{(2, 50)} = 1.33$, $p = .27$), although list recall in the Sleep group tended to be better than in the Wake group ($t_{(33)} = -1.72$, $p = .095$). In order to more sensitively assess recall we adjusted it to the individual's encoding performance, i.e., we expressed list recall as the difference from the individual's word-pair recall at the immediate recall test (set to 100 %) serving as an approximate baseline. Indeed, this measure revealed a pronounced enhancing effect of sleep vs. wakefulness on list recall ($F_{(2,50)} = 3.89$, $p = .03$; $t_{(33)} = 2.64$, $p = .01$, for Sleep vs. Wake group, Figure 1C).

To disentangle the effects of sleep on word-pair recall and list recall, we separately analyzed the effects for recall trials (i) on which both word-pair recall *and* list recall was correct (integrated memory for the item bound into its source) (ii), on which word-pair recall *or* list recall was correct, (iii) on which only word-pair recall was correct but not list recall, and (iv) on trials where only list recall but not word-pair recall was correct, with all of the measures adjusted to the individual's immediate word-pair recall. Unexpectedly, sleep did not significantly enhance recall for word-pairs together with the list in which the pairs occurred (i.e., the item bound into its source, $F_{(2, 50)} = 0.73$, $p = .49$, for main effect Condition; $t_{(33)} = -1.25$, $p = .22$, for Sleep vs.

Wake group, Figure 1D). By contrast, a large beneficial effect was revealed for sleep on general

recall of word-pairs or the correct list (i.e., any of item or source, bound and unbound, $F_{(2, 50)} =$

6.13, $p = .004$; $t_{(33)} = - 3.35$, $p = .002$, for Sleep vs. Wake group, see Figure 1E). This sleep effect

did not appear to be driven by correct word-pair recall for which list recall was incorrect ($p = .56$,

for Sleep vs. Wake group, Figure 1F), but rather by trials with correct list recall but incorrect

recall of the target word (i.e., word-pair, $t_{(31.71)} = -2.24$, $p = .032$, for Sleep vs. Wake, Figure 1G).

Forgetting from the short 1-hour retention interval (Pre-sleep) to the long 11-hour

retention intervals (Sleep, Wake) occurred at a significant level only for the trials with correct

word-pair recall but incorrect list recalls ($p = .023$ and $p = .016$ for comparison with Sleep and

Wake, respectively, Figure 1F). Forgetting was not significant for trials with only correct list

recall (both ps > .26, Figure 1G).

Correlational analyses did not reveal any strong and significant association between

delayed recall of word-pairs and list recall in any of the groups (all rs < .36, all ps > .14),

indicating that – in all experimental groups – both types of recall were largely independent.

Furthermore, correlational analyses revealed that word-pair recall at the immediate recall test

was associated with later list recall across the three retention conditions ($r = .27$, $p = .048$)

suggesting these measures share a component of "general memory capabilities". Such shared

component can be taken to justify our use of immediate word-pair recall values (as an estimate of

memory encoding) for adjusting the individual's list recall (see above).

Correlations between memory performance and sleep parameters

Sleep in children showed the expected pattern with long overall duration and remarkably great

amounts of slow wave sleep (Supplementary Table 1). Of the correlations calculated between

sleep parameters and memory performance, only a few remained significant after correcting for

multiple testing. Recall of word-pairs with simultaneously correct list recall (adjusted to the individual's encoding performance) correlated positively with the percentage of SWS ($r = .61$, $p = .009$) and negatively with the percentage of Stage 2 sleep ($r = -.61$, $p = .01$). Spindle density during NonREM sleep correlated negatively with general word-pair memory ($r = -.59$, $p = .012$) and recall of word-pairs without correct list recall ($r = -.58$, $p = .014$).

Comparison of the temporal dynamics of memory between children and adults

To explore if the forgetting dynamics across the short 1-hr and long 11-hr retention intervals in children differed from those in adults, we tested two groups of adults on the respective Pre-Sleep and Sleep conditions. To account for differences in general learning capabilities between children and adults, we used longer lists in the adults, and age groups were compared based on the percentages of recalled word-pairs (at the different time points) with reference to the total number of word-pairs per list (see Methods). Also here, in the sub-analyses, we refrained from adjusting recall after 1 and 11 hr to immediate recall performance.

Indeed, the percentage of recalled word-pairs at immediate recall did not differ between age and retention groups ($p = .19$ for the main effect of Age, and $p = .54$ for the main effect of Pre-Sleep vs. Sleep group, Figure 2A). Children showed no forgetting of word-pairs at the 1-hour recall and increased forgetting after 11 hours ($p = .034$), whereas adults showed substantial forgetting already at the 1-hour recall with no further increase at the 11-hour recall ($F_{(1,67)} = 5.29$, $p = .025$, for Age $\times$ Pre-sleep/Sleep interaction, Figure 2B). Notably, in the sub-analyses this differential forgetting dynamics in children was only present for the trials with correct word-pair recall in conjunction with incorrect list recall ($F_{(1,67)} = 4.94$, $p = .03$, for Age $\times$ Pre-sleep/Sleep, Figure 2D) but not in any other subgroup of trials, including the trials with both correct word-pair and list recall ($p > .49$, Figure 2C). In fact, the number of trials with both correct word-pair

and list recall was generally less in children than adults ($F_{(1,67)} = 9.45$, $p = .003$, Figure 2C), and the number of trials with correct word-pair recall in conjunction with incorrect list recall was generally better in children than adults ($F_{(1,67)} = 6.97$, $p = .01$, Age main effects).
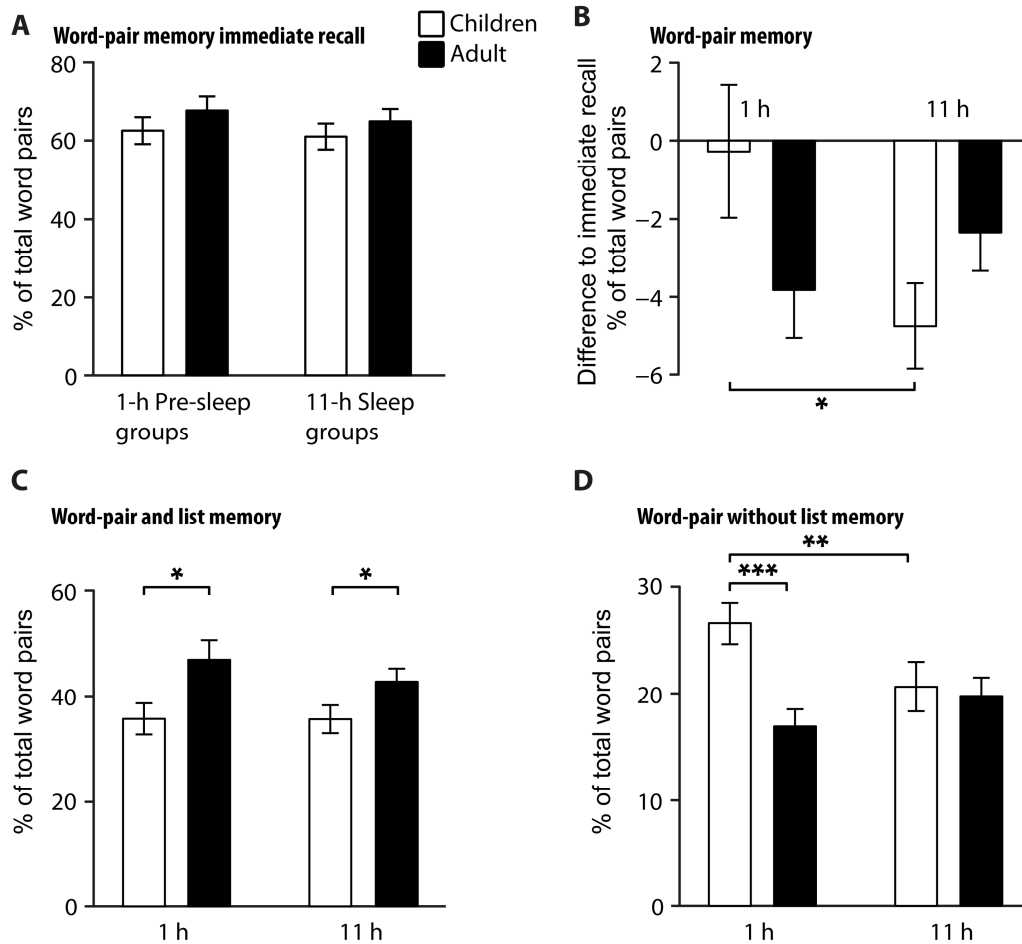
Figure 2. Comparison of recall performance between children (white bars) and adults (black) for the 1-hour Pre-sleep condition (left bars) and 11-hour Sleep conditions (right bars) for (**A**) immediate cued recall of word-pairs during the encoding phase (**B**) delayed cued recall of word-pairs (expressed as difference to immediate recall as in A), and for subgroups of trials (**C**) with both correct word-pair and list recall and (**D**) with correct word-pair but incorrect list recall. Recall is expressed as the percentage of total number word-pairs presented at the encoding phase (40 in children, 80 in adults). Note, recall in C and D is not adjusted to the individual's immediate recall during the encoding phase. * $p < .05$, ** $p < .01$, for pairwise comparisons.

Discussion

We used a modified version of the word paired-associate learning task to determine the extent to which sleep's enhancing effect on word-pair memories in children might originate from a strengthening of episodic representations. Compared to wakefulness, post-learning sleep enhanced word-pair recall, in general, which replicates several previous studies in children (Potkin & Bunney, 2012; Wilhelm et al. 2008) and underlines the robustness of the effect that emerged despite the necessary task changes in comparison to other studies (one-time encoding of word-pairs, encoding of 2 different lists 1 hour apart). Sleep also benefited general list memory as well as isolated list memory (i.e., trials with incorrect word-pair recall). Surprisingly, however, no sleep benefit was revealed for the combined word-pair with correct list memory.

Assuming that correct word-pair memory with correct list recall is a measure closely reflecting the item-source binding characteristic of episodic memory, the absence of any enhancing effect of sleep on this measure argues against the view that sleep effects on episodic representations essentially contribute to the general enhancement in word-pair memory, all the more so since both measures of memory performance were uncorrelated. The absence of a sleep-induced enhancement in combined word-pair/list recall also diverges from previous findings indicating a sleep-induced enhancement of episodic "what-where-when" memory in children of the same age group, although in that study the gain in episodic memory after sleep was not superior to that seen in adults (Wang et al., 2016). A tentative explanation for this discrepancy is that unlike in that foregoing study manipulating spatial as well as temporal context aspects of the episode, here, source memory was mainly defined by the temporal context aspects, i.e., by the second list learned 1 hour after the first list. Temporal features of episodic memory formation show a protracted development well into adolescence (e.g., Picard et al., 2012).

Indeed, the observations of absent or only moderate sleep-induced enhancements in measures of episodic memory in this and previous studies might simply reflect the immaturity of the episodic memory system and the fact that at this age children's encoding and forming memories for episodes is less well structured in time and space (Ghetti & Bunge, 2012; Riggins, 2014). Supporting this view, the comparison of memory dynamics with an adult control group revealed generally reduced memory for word-pairs in conjunction with the correct list, but enhanced memory for word-pairs in the absence of correct list recall, i.e., children appear to preferentially store word-pair memories unbound to their source. On the other hand, correlational analyses confirmed that like in adults (Inostroza & Born, 2013), SWS in children preferentially supports episodic-like memory, here of word-pairs bound to the correct list. In this context, the strong negative correlation of EEG spindle density with word-pair memory in the absence of list recall and with general word-pair recall, was unexpected and also diverges from findings in adults of a link between spindles and non-episodic semantic types of memory (e.g., Schabus et al., 2004). It might point to differential functions of sleep spindles for memory processing in children.

Apart from enhancing general word-pair memory, sleep in the children also generally enhanced list memory, as well as isolated list memory (in the absence of correct word-pair memory). The result of particularly strong effects of sleep on isolated list memory is a further hint that sleep in children does not act towards enhancing episodic memory features binding source with item characteristics. In fact, sleep-induced enhancements in word-pair and list memories that are entirely independent of whether or not respective source or item information is also correctly recalled, could be taken to speculate that sleep in children fosters the "unbinding" of item and source information in episodic representations. Sleep unbinding episodic

representations has been observed in adults although often developing more slowly over several nights (Cairney et al., 2011; Deliens & Peigneux, 2014; Jurewicz, Cordi, Staudigl, & Rasch, 2016; Sweegers & Talamini, 2014). That children overall form less distinct episodic memory, in this context, might explain unbinding effects to emerge faster in the children (already after one night), and might also explain the strong enhancement in list memory considered incidentally encoded source information, as encoding in children would be expected to be less distinctive between source and item information. However, the hypothesis of a fast unbinding effect of sleep on episodic memory in children, although attractive, needs to be scrutinized using task designs directly testing the context-dependency of item recall after sleep.

Acknowledgements

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Supplementary material

Sleep recordings and EEG analysis

Sleep was recorded using standard polysomnography including EEG recordings from Fz, F3, F4, Cz, C3, C4, Pz, P3, and P4 electrode sites (reference: linked electrodes at the mastoids, ground: Fpz), electromyography on the chin (musculus mentalis), and electrooculography (around the eyes). Signals were amplified (BrainAmp, Brain Products, Gilching, Germany), digitized (sampling rate >250 Hz) and filtered (EEG and EOG 0.3–35 Hz, EMG 10–100 Hz). Sleep stages were scored offline by two experienced raters according to standard criteria (Rechtschaffen & Kales, 1968). Concrete slow oscillations and general spindles (9–15 Hz) were detected using standard settings of the SpiSOP tool (Weber, 2016) which was based on previously published algorithms (Mölle, Marshall, Gais, & Born, 2002), and spindle and slow oscillation parameters (e.g. density) were averaged across the anterior-posterior axis, i.e., Fz, Cz and Pz. Supplementary Table 1 contains a summary of the sleep scoring parameters. Due to exclusion of poor performers (see Methods) correlation analysis of sleep scoring, spindle and slow oscillation parameters with overnight changes in memory (as presented in Figure 1) included one Sleep group child less than was usable from the EEG sleep recordings (n = 17).

Supplementary Table 1

Sleep parameters (*n* = 18)

Stages		
TST [min]	553.19 ±	3.45
Sleep onset [min]	15.80 ±	3.97
WASO [%]	0.25 ±	0.16
Stage 1 [%]	2.07 ±	0.18
Stage 2 [%]	21.66 ±	1.61
SWS [%]	49.89 ±	1.97
NonREM [%]	71.56 ±	1.04
REM [%]	25.07 ±	0.86

Means ± SEM are shown for the total sleep time (TST), sleep onset (with reference to the time of lights off and beginning of first occurrence of stage 1-sleep epoch followed by stage 2-sleep), and time spent awake after sleep onset (WASO), sleep stage 1, sleep stage 2, SWS, NonREM (S2 + SWS) and REM in percentage of total sleep time.

Children's vigilance and subjective tiredness

Before the encoding of each list and before retrieval vigilance was assessed using reaction time performance during a 5-min version of the Psychomotor Vigilance Task (PVT). Furthermore, subjective tiredness was assessed using the Stanford Sleepiness Scale (SSS, Hoddes, Dement, & Zarcone, 1972).

Reaction time on the PVT did not differ between Pre-sleep, Sleep, and Wake conditions, neither at encoding (Pre-sleep: 403.56 ± 13.84 ms, Sleep: 420.64 ± 33.44 ms, Wake: 390.21 ± 15.27 ms) nor at retrieval (Pre-sleep: 449.04 ± 32.48 ms, Sleep: 391.35 ± 16.03 ms, Wake: 408.69 ± 19.44 ms, all $p > .22$). Subjective tiredness (as assessed by the SSS) of the Pre-sleep group was lower than the other two groups at encoding (Pre-sleep: 1.58 ± 0.14, Sleep: 2.63 ± 0.30, Wake: 2.61 ± 0.30, $p < .007$ for both comparisons) but did not differ between the groups at retrieval testing (Pre-sleep: 1.78 ± 0.17, Sleep: 2.41 ± 0.26, Wake: 2.36 ± 0.34, $p > .19$). Considering SSS at retrieval or encoding as covariate did not essentially change any of the reported ANOVA effects for memory recall except that the decrease in general word-pair memory from the Pre-sleep to the Sleep condition in children failed to reach significance (Figure 2A). This suggests the reduced forgetting in the Pre-sleep children might be partially driven by lower tiredness at encoding in this condition. Otherwise, these results exclude that sleep-wake related differences in memory recall were confounded by non-specific alterations in vigilance.

Statistical Analysis

Statistical analysis was done using [*R*] (Mac OS X version 1.7.1, R Core Team, 2012). Mean ± SEM are reported. Kruskal-Wallis one-way ANOVA was used as nonparametric test in case normality and homogeneity assumptions of ANOVA were not met. *Post-hoc* tests followed significant ANOVAs effects, including Student's t-Test for equal variances and Welch's t-Test with approximation to the degrees of freedom for unequal variances; otherwise we used nonparametric Mann–Whitney *U* test. Cohen's *d* indicated central effect sizes. Moreover, associations were tested using linear regression analysis with Pearson product-moment and Spearman´s rank correlation for the parametric and nonparametric tests, respectively. For simplicity, *p*-values are reported uncorrected for multiple comparisons. Significance level was set to 0.05.

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