Research

The effect of zolpidem on targeted memory reactivation during sleep

Julia Carbone,[1,2] Carlos Bibian,[1,2] Patrick Reischl,[1] Jan Born,[1,3,4] Cecilia Forcato,[5,6] and Susanne Diekelmann[1,7]

[1]Institute of Medical Psychology and Behavioural Neurobiology; [2]Graduate Training Centre of Neuroscience, International Max Planck Research School; [3]Werner Reichardt Centre for Integrative Neuroscience, University of Tübingen, Tübingen, Germany; [4]Institute for Diabetes Research and Metabolic Diseases of the Helmholtz Center Munich at the University of Tübingen, Tübingen, Germany; [5]Laboratorio de Sueño y Memoria, Depto. de Ciencias de la Vida, Instituto Tecnológico de Buenos Aires (ITBA), Buenos Aires, Argentina; [6]Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Buenos Aires, Argentina; [7]Department of Psychiatry and Psychotherapy, University Hospital Tübingen, Tübingen, Germany

According to the active system consolidation theory, memory consolidation during sleep relies on the reactivation of newly encoded memory representations. This reactivation is orchestrated by the interplay of sleep slow oscillations, spindles, and theta, which are in turn modulated by certain neurotransmitters like GABA to enable long-lasting plastic changes in the memory store. Here we asked whether the GABAergic system and associated changes in sleep oscillations are functionally related to memory reactivation during sleep. We administered the $GABA_A$ agonist zolpidem (10 mg) in a double-blind placebo-controlled study. To specifically focus on the effects on memory reactivation during sleep, we experimentally induced such reactivations by targeted memory reactivation (TMR) with learning-associated reminder cues presented during post-learning slow-wave sleep (SWS). Zolpidem significantly enhanced memory performance with TMR during sleep compared to placebo. Zolpidem also increased the coupling of fast spindles and theta to slow oscillations, although overall the power of slow spindles and theta was reduced compared to placebo. In an uncorrected exploratory analysis, memory performance was associated with slow spindle responses to TMR in the zolpidem condition, whereas it was associated with fast spindle responses in placebo. These findings provide tentative first evidence that GABAergic activity may be functionally implicated in memory reactivation processes during sleep, possibly via its effects on slow oscillations, spindles and theta as well as their interplay.

Sleep supports the consolidation of newly acquired memories (Mednick et al. 2011; Klinzing et al. 2019). According to the active system consolidation theory, new memories and their associated neuronal activation patterns become spontaneously reactivated (replayed) following learning in the sleeping brain (Wilson and McNaughton 1994; Diekelmann and Born 2010). These reactivations allow for the redistribution and integration of the memory representations from hippocampal to neocortical sites for long-term storage (Rasch and Born 2007; Klinzing et al. 2019). Memory reactivation during sleep has been proposed to rely on the synchronized interplay of electrophysiological oscillations characteristic of non–rapid eye movement (NREM) sleep, mainly neocortical slow oscillations (SOs, <1 Hz), thalamocortical spindles (9–15 Hz), and hippocampal ripples (80–200 Hz) (Mölle et al. 2009; Staresina et al. 2015; Helfrich et al. 2018; Ngo et al. 2020). Particularly, sleep spindles and their intricate phase coupling to SO have been suggested to be mechanistically involved in memory consolidation processes during sleep (Ulrich 2016; Antony et al. 2019). It has been proposed that memories become reinstated by spindle events, specifically during the up-state of slow oscillations, allowing for the flow of information between different brain sites as well as the induction of lasting structural and functional plastic changes in the learning-associated neuronal networks (Rosanova and Ulrich 2005; Peyrache and Seibt 2020). In addition to sleep spindles, neocortical and hippocampal theta activity (4–8 Hz) is also phase-locked to SO during NREM sleep (Gonzalez et al. 2018; Cox et al. 2019; Krugliakova et al. 2020), and this coupling has been related to memory consolidation during sleep (Schreiner et al. 2018).

A number of neuromodulators seem to be involved in the generation of sleep spindles, SO and associated memory processing, most notably GABA (gamma-aminobutyric acid), which is the major inhibitory neurotransmitter (Lancel 1999; Ulrich et al. 2018). Sleep spindles and sleep-dependent memory processing can be boosted by targeting the GABAergic system pharmacologically (Mednick et al. 2013). Zolpidem is one of the most frequently used drugs in this regard, binding to $GABA_A$ receptors at the same location as benzodiazepines, thereby acting as a $GABA_A$ receptor agonist (Lemmer 2007). Zolpidem increases the time spent in slow-wave sleep (SWS) and reduces the amount of rapid eye movement (REM) sleep (Kanno et al. 2000; Uchimura et al. 2006; Zhang et al. 2020). Zolpidem also increases the density and power of sleep spindles (Dijk et al. 2010; Lundahl et al. 2012; Mednick et al. 2013; Niknazar et al. 2015; Zhang et al. 2020) as well as the coupling of spindles to SO (Niknazar et al. 2015; Zhang et al. 2020), and it was further found to enhance declarative memory

Corresponding author: susanne.diekelmann@uni-tuebingen.de
Article is online at http://www.learnmem.org/cgi/doi/10.1101/lm.052787.120.

consolidation during sleep, with postsleep performance improvements being associated with higher spindle density and spindle power as well as with SO–spindle coupling (Kaestner et al. 2013; Mednick et al. 2013; Zhang et al. 2020).

However, it remains unclear whether the changes in sleep stages, sleep spindles, and SO–spindle coupling after pharmacological manipulation with zolpidem are functionally related to the mechanisms underlying sleep-dependent memory consolidation such as memory reactivation. Over the last few years, targeted memory reactivation (TMR) has been increasingly applied to manipulate memory reactivation during sleep experimentally by presenting learning-associated reminder cues like odors or sounds (Oudiette and Paller 2013; Hu et al. 2020; Klinzing and Diekelmann 2020). TMR biases sleep-related neuronal replay events toward the reactivated memory contents (Lewis and Bendor 2019) and enhances subsequent recall performance (Rudoy et al. 2009; Diekelmann et al. 2011; Schreiner et al. 2015; Cairney et al. 2018). Although a few studies observed modulations of SOs (Rihm et al. 2014), sleep spindles (Cox et al. 2014), and SO–spindle coupling (Bar et al. 2020) with TMR during sleep, studies on the role of specific neurotransmitters and particularly on the role of GABAergic neurotransmission and associated changes in sleep oscillations for targeted memory reactivation are entirely lacking. One previous study tested the effect of pharmacologically increased GABAergic activity by administering the benzodiazepine clonazepam after cued reactivation of a declarative memory during wakefulness (Rodríguez et al. 2013). Clonazepam increased memory performance when it was administered after reactivation with an incomplete reminder cue, suggesting that increasing GABAergic neurotransmission may enhance the restabilization of reactivated declarative memories in humans during wakefulness.

In the present study, we tested the effect of modulating GABAergic activity with zolpidem on targeted memory reactivation during sleep and associated changes in sleep spindles as well as SO–spindle and SO–theta coupling. We hypothesized that zolpidem enhances the beneficial effects of targeted memory reactivation on memory performance and that this enhancement is associated with increases in spindle density, spindle power, SO–spindle coupling, and possibly SO–theta coupling, and the amount of SWS. Participants were trained on a memory task including 30 sound–word associations in the evening (Forcato et al. 2020) and received an oral dose of 10 mg zolpidem ($n = 11$) or placebo ($n = 11$) after training before a full night of sleep in the sleep lab (Fig. 1). During the night, incomplete reminder cues (sounds + first syllable of the associated words) were played again via in-ear headphones during SWS. The next morning, participants were trained on an interference memory task to probe the stability of the original memory, which was tested 30 min later.

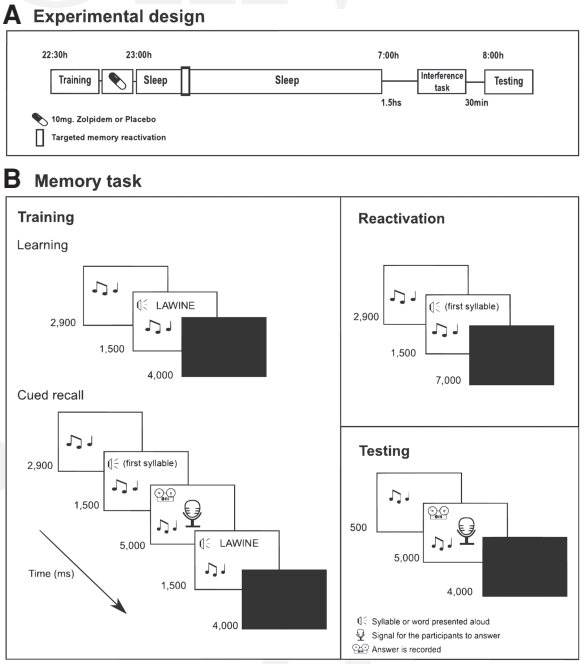

Figure 1. Experimental design and memory task. (*A*) All subjects took part in a training session at ~22.30, were administered with placebo ($n = 11$) or 10 mg zolpidem ($n = 11$) before going to bed at 23:00, and received targeted memory reactivation during the first SWS period. After ~8 h of sleep, in the morning, subjects learned an interference task and were tested on the original memory task in a testing session 30 min after the interference task. (*B*) Training: First, subjects were presented with 30 sound–word associations for learning. For each association, the sound was presented first for 2900 msec. The sound then continued accompanied by the word written on the screen and spoken aloud for 1500 msec. After a 4000-msec break, the next association was presented in the same way. After all associations were presented once, participants completed an immediate cued recall test. For each association, the sound was presented for 2900 msec. The sound then continued accompanied by the first syllable of the associated word for 1500 msec. Participants were then given 5000 msec to say the complete word aloud (sound continued during the entire period). Independently of their response, the correct answer was then presented on the screen and via headphones for 1500 msec. Reactivation: Each sound was first presented alone for an average of 2900 msec; the sound then continued accompanied by the first syllable of each word for another 1500 msec. After a 7000-msec break, the next sound–syllable pair was presented until all 30 pairs had been presented once. Testing: Each sound was presented for 500 msec and then the sound continued and subjects had 5000 msec to say the associated word aloud. After a break of 4000 msec, the procedure continued for the rest of the 30 associations. Adapted from Forcato et al. (2020).

Results

Memory performance

Memory reactivation with sound–syllable reminders during SWS led to a significantly better memory performance in the zolpidem group compared to the placebo group ($F_{(2,20)} = 1.17$, $P = 0.034$) (Fig. 2). Memory performance was calculated as memory change by subtracting the amount of correct responses during training from the amount of correct responses at testing, indicating less forgetting with zolpidem than with placebo (-1.09 ± 0.90 vs. -4.81 ± 1.36). Both groups were comparable in initial learning of the memory task during training ($P = 0.97$) as well as in learning of the interference task before testing ($P = 0.41$; Table 1). Subjective sleepiness (assessed with the Stanford Sleepiness Scale) did not differ between groups, neither at training nor at interference learning or testing (all $P > 0.15$; Table 1). To control for possible awareness of the sound–syllable reminders during sleep, in the morning all participants were asked to indicate whether they had heard any of the reminders during sleep. The number of recognized reminders was very low and did not differ between groups ($P = 0.60$; Table 1).

Sleep data analyses in the whole night

Sleep architecture

The zolpidem group tended to show more stage 3 sleep ($P = 0.053$) and overall more SWS than the placebo group ($P = 0.075$; Table 2). Total sleep time, time awake, time in stage 1, stage 2, stage 4, and REM sleep were comparable between both groups ($P > 0.15$). There were no significant correlations between time spent in the different sleep stages and memory performance (all $P > 0.10$, corrected as well as uncorrected).

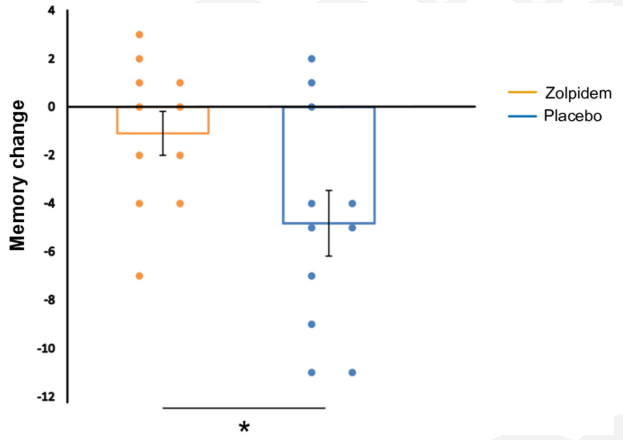

Figure 2. Zolpidem enhances memory performance with targeted memory reactivation. Memory change refers to the number of correct responses at testing minus training, indicating less forgetting in the zolpidem group (orange) compared to placebo (blue). Means ± SEM are shown. (*)$P < 0.05$.

Sleep spindles

When analyzing all epochs of stage 2 and SWS separately for the whole night, the zolpidem and placebo groups showed no differences in spindle count, spindle density, and spindle power, neither for slow spindles (9–12 Hz) nor fast spindles (12–15 Hz), nor for power in the total spindle (i.e., sigma) range (9–15 Hz) (all $P > 0.10$, corrected for multiple comparisons). When inspecting the uncorrected data for exploratory purposes, there was only one difference in slow spindle count, with the zolpidem group showing higher numbers of slow spindles at parietal sites during SWS compared to placebo (219.8 ± 38.3 vs. 124.5 ± 16.8; $P = 0.04$ uncorrected). There were no significant correlations of any of these parameters with memory performance (all $P > 0.10$, corrected and uncorrected).

Slow oscillations and delta power

Slow oscillations (SOs, 0.5–1 Hz) count, density, and power did not differ between the zolpidem group and the placebo group, neither in stage 2 nor during SWS at any of the electrode sites (all $P > 0.10$, corrected and uncorrected). Likewise, there were no significant differences between the zolpidem and placebo groups for power in the delta frequency band (1–4 Hz) (all $P > 0.10$, corrected and uncorrected), and there were no significant correlations for any of the SO parameters and delta power with memory performance (all $P > 0.10$, corrected and uncorrected).

Theta power

There were no significant differences between the zolpidem and placebo groups for theta power (4–8 Hz) in stage 2 as well as during SWS at any of the electrode sites (all $P > 0.10$, corrected and uncorrected). There were no correlations between any of the power values and memory performance (all $P > 0.30$, corrected and uncorrected).

Time course of spindle and theta power across the night

Based on previous evidence that zolpidem may affect spindle and theta power during sleep (Zhang et al. 2020), we conducted an additional exploratory analysis examining the time course of spindle and theta power across the night in relation to targeted memory reactivation and the active drug effect of zolpidem. We plotted the average power values for slow spindle, fast spindle, and theta power in all 30-sec epochs of SWS and compared these values between the zolpidem and placebo groups (Fig. 3). After correction for multiple comparisons, there were no significant differences, presumably because of the large number of comparisons (i.e., 170 single epochs of SWS). For exploratory purposes, we inspected the uncorrected data and observed that slow spindle power was constantly lower in the zolpidem group within the first 113 epochs of SWS (all $P < 0.05$, uncorrected). This interval corresponds roughly to 2.5 h of sleep and the expected maximum drug effect of zolpidem (with a half-life of 2–3 h). There was no difference between groups during the drug's half-life for fast spindles (all $P > 0.15$, uncorrected); however, fast spindle power was transiently reduced shortly after the drug's half-life during epochs 109–121 (all $P < 0.05$, uncorrected). The time course of theta power was similar to that of slow spindle power, with reduced power in the zolpidem group during the first 55 epochs of SWS (all $P < 0.05$, uncorrected). To circumvent the problem of a large number of comparisons when examining single epochs of SWS, in an additional analysis we grouped all epochs into four sleep cycles across the night (similar to quartiles in Zhang et al. 2020). In this analysis, slow spindle power was reduced during SWS in the first cycle ($P = 0.01$), second cycle ($P = 0.052$), and third cycle ($P = 0.014$), after correction for multiple comparisons. Theta power was reduced during the first cycle only ($P = 0.03$, after correction for multiple comparisons), and there were no significant differences for fast spindles (all $P > 0.10$, corrected and uncorrected). There were also no correlations between the average of the significant SWS epochs for each frequency band and memory performance (all $P > 0.10$, corrected and uncorrected).

Coupling of slow oscillations with spindles and theta power

To test whether zolpidem affected the coupling of spindle and theta events to SOs, we calculated a modulation index reflecting the relationship between the phase of the SOs with the amplitude of slow spindles, fast spindles, and theta events, respectively. We did this analysis for total NREM sleep as well as, separately, for NREM sleep during the first 2.5 h of sleep (corresponding to zolpidem's half-life) and the remaining periods of NREM sleep after the first 2.5 h of sleep (i.e., post zolpidem's half-life). The coupling of slow spindles, fast spindle,s and theta with SOs did not differ between the zolpidem group and the placebo group for total NREM sleep (all $P > 0.35$). However, coupling of SOs to spindle and theta

Table 1. Results for the memory task, subjective, and heard/not-heard task

Memory task	Placebo	Zolpidem	*P*
Training	24.9 ± 0.9	25.0 ± 1.1	0.97
Interference	21.7 ± 2.2	23.8 ± 1.1	0.41
Testing	20.1 ± 1.9	24.1 ± 1.4	0.12
Memory change	−4.8 ± 1.4	−1.1 ± 0.9	0.034
Sleepiness			
Training	3.7 ± 0.2	3.3 ± 0.3	0.19
Interference	2.8 ± 0.2	2.7 ± 0.3	0.75
Testing	2.5 ± 0.2	2.1 ± 0.2	0.16
Heard/not-heard	4.9 ± 2.0	3.8 ± 1.8	0.60

Mean number of correct responses is indicated for the memory task ± SEM for the immediate cued recall at training of the original task (Training), the immediate cued recall at training of the interference task (Interference), and the test of the original task (Testing). Memory change indicates the difference between Testing and Training. Ratings of subjective sleepiness in the Stanford Sleepiness Scale (SSS) ± SEM were assessed before each memory test. Values of the "heard/not-heard" task indicate the mean number ± SEM of associations subjects indicated as having heard during sleep (assessed in the morning).

Table 2. Sleep architecture

Sleep stage	Placebo	Zolpidem	*P*
Wake	17.7 ± 7.5	14.6 ± 4.4	0.73
S1	6.6 ± 1.8	5.8 ± 1.8	0.73
S2	306.7 ± 9.1	286.4 ± 11.1	0.17
S3	49.3 ± 4.4	62.8 ± 4.8	0.053
S4	12.6 ± 6.2	25.4 ± 9.1	0.25
SWS	62.0 ± 9.2	88.2 ± 10.5	0.075
REM	79.2 ± 10.0	70.4 ± 6.4	0.46
TST	472.6 ± 8.4	466.2 ± 6.4	0.55

Mean amount of time (in min) spent in the different sleep stages ± SEM for each group and the corresponding *P* values for independent sample *t*-tests. (Wake) time awake after sleep onset, (S1) stage 1 sleep, (S2) stage 2 sleep, (S3) stage 3 sleep, (S4) stage 4 sleep, (SWS) slow-wave sleep (sum of S3 and S4), (REM) rapid eye movement sleep, (TST) total sleep time.

events differed during and after the half-life of zolpidem. Coupling of theta to SO was significantly stronger during the half-life of zolpidem compared to the post-half-life sleep period, particularly in the zolpidem group but not in the placebo group ($P = 0.006$, for interaction zolpidem/placebo × half-life/post-half-life at central electrode positions; Fig. 4A). Post hoc tests revealed enhanced theta–SO coupling during the first 2.5 h of sleep in the zolpidem group ($P = 0.014$) but not in the placebo group ($P = 0.38$). A similar pattern was evident for fast spindle–SO coupling, with stronger coupling during the first 2.5 h of sleep in the zolpidem group ($P = 0.036$) but not in the placebo group ($P = 0.61$; interaction zolpidem/placebo × half-life/post-half-life at frontal electrode positions: $P = 0.076$; Fig. 4A). Slow spindle–SO coupling revealed a trend toward an overall stronger coupling during the first 2.5 h of sleep ($P = 0.057$, for main effect half-life/post-half-life) but this effect was not specific for zolpidem ($P = 0.29$, for interaction zolpidem/placebo × half-life/post-half-life at parietal electrode positions). Figure 4B,C shows the distribution of theta, slow spindle, and fast spindle events in relation to the phase of the SO during and after zolpidem's half-life. From this figure it can be taken that fast spindles mostly occurred at the positive peak (up-state) of the SO, whereas theta and slow spindles were more prominent at the negative peak (down-state). There were no correlations between any of the coupling measures and memory performance (all $P > 0.05$, corrected and uncorrected).

Sleep data analyses in the reactivation phase

To test for the effect of zolpidem on activity patterns during targeted memory reactivation, we conducted time–frequency analyses upon the presentation of the sound–syllable reminders (Fig. 5). First, we looked at the event-related potentials (ERPs) evoked in response to the sound–syllable associations (at central electrode positions). Both the zolpidem and placebo group exhibited a solid response comparable to a SO/K-complex upon the presentation of the syllable (Fig. 5A,B, all trials aligned to syllable onset, $t = 0$). Although the ERP amplitude in the zolpidem group appears to be relatively small, the ERP peak-to-peak amplitude did not differ significantly between zolpidem and placebo ($P = 0.16$).

Consistent with previous findings, we observed power increases in the theta/slow spindle frequency range in the up-to-down transition of the ERP (at ~0.2–1 sec), followed by a fast spindle power increase around the peak of the ERP (at ~1–1.5 sec) in both the zolpidem and placebo groups. When comparing the time–frequency maps of both groups (Fig. 5C), statistical differences were only found in one cluster between –1 and 0 sec in the slow spindle frequency range (9–12 Hz; $P = 0.006$), with the zolpidem group exhibiting a weaker slow spindle response than the placebo group. No other differences reached significance. Additional time–frequency analyses were conducted after realigning the data to sound onset, which revealed essentially the same results.

To test whether the observed responses to reactivation were associated with memory performance, we conducted correlation analyses between relative power values in specific time windows (Fig. 6A) and memory performance. After correction for multiple comparisons, there were no significant correlations. However, when inspecting the uncorrected data for exploratory purposes, in the zolpidem group, better memory performance was associated with higher slow spindle power in an early-pre-cue time window (−1.4 to 0 sec; for central electrodes: $P = 0.038$, uncorrected; Fig. 6B). Interestingly, this time window corresponds to the significant cluster of reduced slow spindle power responses in the zolpidem group in the time–frequency analysis. In the placebo group, better memory performance was correlated with fast spindle power in a late-pre-cue time window (−2.8 to −1.4 sec; for central electrodes: $P = 0.021$, for parietal electrodes: $P = 0.022$, uncorrected; Fig. 6C; all other $P > 0.05$, corrected and uncorrected). There were no significant correlations between memory performance and theta power (all $P > 0.15$, corrected and uncorrected).

Discussion

In the present study, we examined the functional role of GABAergic neurotransmission and associated sleep oscillations as potential mechanisms underlying the benefical effects of targeted memory reactivation (TMR) during sleep. Our findings provide tentative first evidence that the $GABA_A$ agonist zolpidem may enhance memory consolidation with TMR, facilitating the

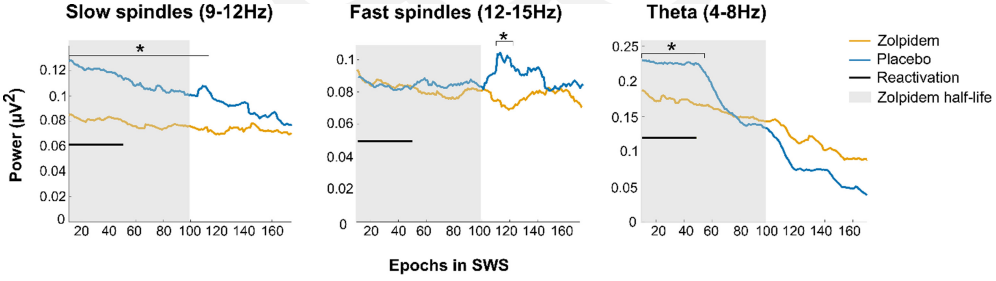

Figure 3. Time course of spindle and theta power with zolpidem during SWS. In an exploratory analysis, zolpidem decreases power in the slow spindle and theta band during zolpidem's half-life. Fast spindle power is decreased for a short period after the half-life. Average power for each SWS epoch is shown for zolpidem (orange) and placebo (blue). The black line shows SWS epochs with targeted memory reactivation (i.e., presentation of sound–syllable reminders). The gray shaded area represents the expected drug effect of zolpidem (i.e., its half-life of 2–3 h). * SWS epochs with significant differences in power values between the zolpidem and placebo group without correction for multiple comparisons ($P < 0.05$). Please note that, because of the large number of comparisons, significant differences do not survive corrections for multiple comparisons.

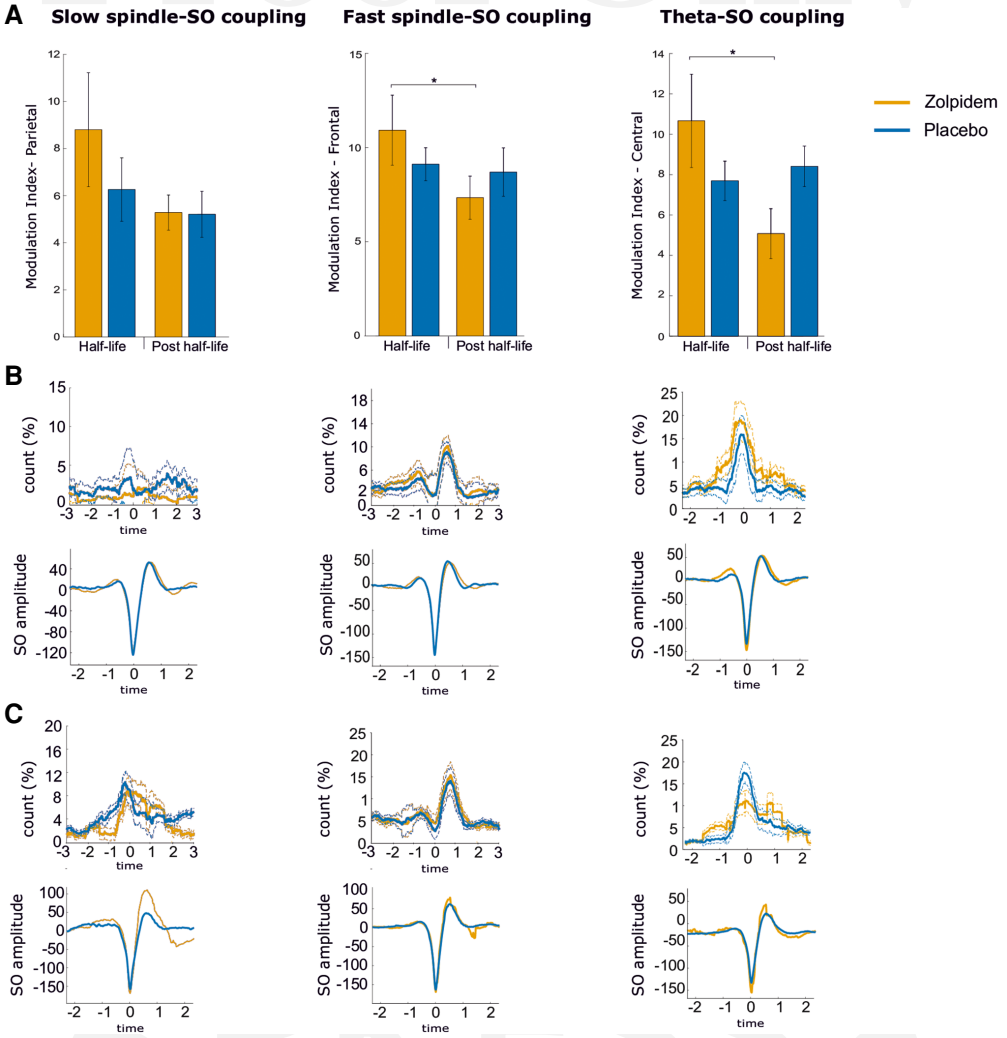

Figure 4. Zolpidem enhances the coupling of spindles and theta to slow oscillations. (*A*) Coupling between the phase of slow oscillations and the amplitude of fast spindles and theta (as expressed in the modulation index) was higher during zolpidem's half-life (i.e., NREM sleep during the first 2.5 h of sleep, "half-life") compared to the sleep period after the first 2.5 h ("post-half-life") in the zolpidem group. The *lower* graphs indicate peri-event histograms of slow spindle, fast spindle, and theta event counts as well as slow oscillation (SO) amplitudes for slow spindle–SO, fast spindle–SO, and theta–SO coupling (*B*) during NREM sleep of the first 2.5 h of sleep (i.e., "half-life") and (*C*) during NREM sleep after the first 2.5 h sleep period (i.e., "post-half-life"). These data show the preferred phase of the SOs at which theta, slow spindles, and fast spindles occurred. Data for zolpidem (orange) and placebo (blue) are indicated for parietal electrode sites for slow spindle–SO coupling, frontal electrode sites for fast spindle–SO coupling, and central electrode sites for theta–SO coupling. Means ± SEM are shown. (*) $P < 0.05$.

stabilization of externally reactivated memories. We further found specific changes in reactivation-related responses after zolpidem administration, particularly a reduction in slow spindle power, with a lower reduction being in turn associated with better memory performance. Additional exploratory analyses revealed a general reduction of slow spindle and theta power, but at the same time a stronger coupling of fast spindles and theta events to slow oscillations, with both effects being particularly pronounced during the first 2–3 h of sleep, corresponding to the expected maximum drug effect of zolpidem. Interestingly, these zolpidem-induced changes in oscillatory patterns were not associated with memory performance.

The finding that pharmacologically modulating GABAergic neurotransmission with zolpidem facilitates the memory benefits of TMR is in line with previous evidence on beneficial effects of zolpidem on spontaneous memory consolidation during sleep. Zolpidem was found to increase memory consolidation during sleep for emotional picture recognition (Kaestner et al. 2013) and word-pair associations (Mednick et al. 2013; Niknazar et al. 2015; Zhang et al. 2020). Here we show improved memory performance after zolpidem administration in a sound–word association task. However, our findings go beyond this previous research in two important regards. Previous studies exclusively tested the effect of zolpidem on spontaneous memory consolidation during sleep, and it is commonly assumed that this consolidation relies on the reactivation of memory representations during sleep. In the present study, we manipulated memory reactivation experimentally with auditory reminder cues, thereby systematically testing the role of zolpidem administration and associated changes in sleep oscillations for memory reactivation during sleep. In a previous study from our group, we have shown that the same TMR protocol with incomplete reminders (i.e., sounds + first syllable of the associated word) facilitated memory stabilization after 40 min and 8 h of sleep (Forcato et al. 2020). Another study, testing memory

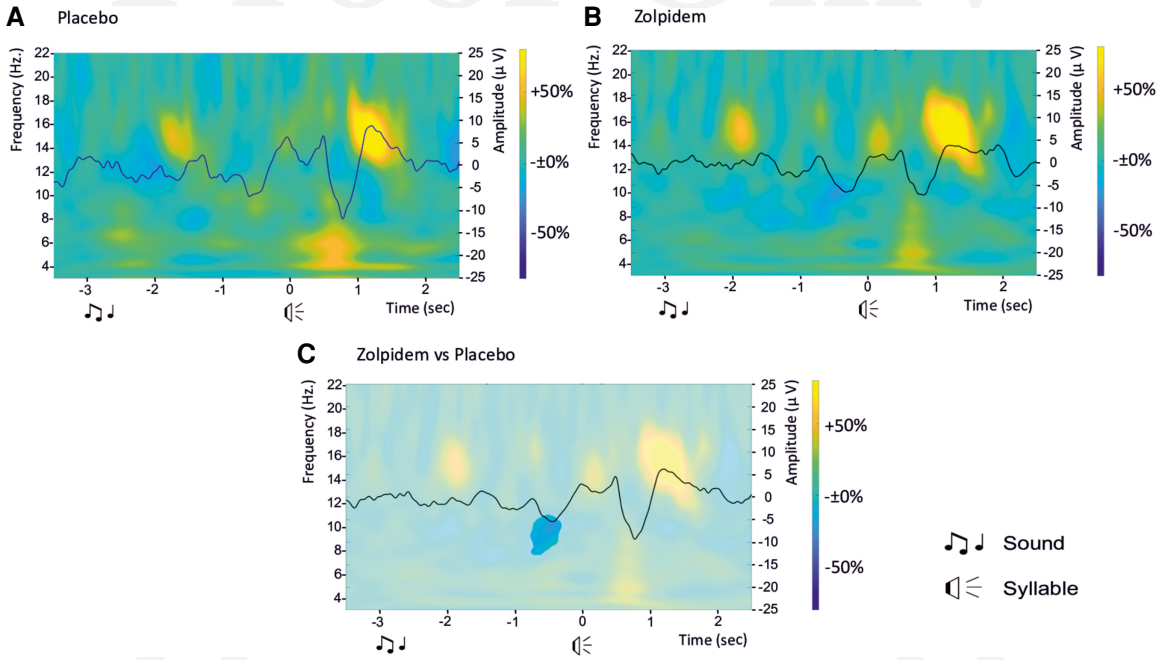

Figure 5. Time–frequency analyses of targeted memory reactivation. Time–frequency representation for (*A*) placebo and (*B*) zolpidem, each with their corresponding ERP. The color maps show power changes relative to a baseline of 1 sec right before sound onset (i.e., −4 to −3 sec). (*C*) Comparison between responses in the zolpidem and placebo groups, with ERPs averaged across both groups. The mask shows the only significant cluster (in blue), indicating lower power in the slow spindle frequency band (9–12 Hz) for the zolpidem group at around −0.5 sec (i.e., about 2.5 sec after sound onset) ($P < 0.05$).

reactivation during wakefulness, observed better memory performance following the modulation of GABAergic activity with clonazepam after reactivation with incomplete reminders (Rodríguez et al. 2013). In combination with the present findings, this evidence suggests that the GABAergic system is functionally involved in memory reactivation and stabilization during wakefulness and sleep. However, the exact mechanisms of GABAergic neurotransmission for memory functions are not well understood, with previous evidence partly observing conflicting results. For instance, a previous study from our group found that nonspecifically increasing the availability of GABA by administering the GABA reuptake-inhibitor tiagabine did not improve declarative memory consolidation during sleep and even reduced spindle–SO phase coupling (Feld et al. 2013). It could be speculated that the beneficial effects of GABAergic neurotransmission for memory reactivation and consolidation depend on the phasic activation of the $GABA_A$ receptor, as observed with the $GABA_A$ agonist zolpidem, instead of a tonic activation through the generally increased availability of GABA following the administration of GABA reuptake inhibitors (Lancel 1999). It is well-known that GABA receptors are located widespread in the brain, particularly in areas that are implicated in memory processes such as the hippocampus and the amygdala (Izquierdo and Medina 1991; Chapouthier and Venault 2002; Heaney and Kinney 2016). Thereby the actions of GABA and different GABA receptors with their single subunits may exert differential effects on synaptic plasticity, learning, and memory (Collinson et al. 2002), possibly also depending on the interaction with other cotransmitters like neuropeptide Y (Comeras et al. 2021). The exact neurophysiological mechanisms of the GABAergic system and its involvement in memory formation go beyond the aim of the present study and should be subject to further investigation.

With regard to the effects of zolpidem on sleep architecture, we observed a trend toward more time spent in SWS, which is in keeping with previous reports of increased amounts of SWS following zolpidem administration (Kaestner et al. 2013; Mednick et al. 2013; Zhang et al. 2020). Contrary to our hypothesis, we did not observe a significant increase in spindle density or spindle power in the zolpidem group compared to placebo, even though this effect has been frequently reported in previous studies (Brunner et al. 1991; Zhang et al. 2020). For instance, Mednick and colleagues observed an increase in the density of slow and fast spindles during a nap with zolpidem, with more spindles also being associated with memory improvements (Mednick et al. 2013). Yet, some studies did not observe an increase in spindle density (e.g., Zhang et al. 2020). Although the slow spindle frequency range was not explicitly analyzed in the study by Zhang and colleagues, it can be observed from the power spectra that there was a reduction of power in lower frequency ranges after administration of zolpidem (Zhang et al. 2020), with parts of this frequency range overlapping with the range that was defined as slow spindle range in the present study. This reduction corresponds with the decrease of slow spindle power observed in the present study. Interestingly, decreased power in the slow spindle frequency range was particularly evident during the first 2–3 h of sleep in the present study, that is, the maximum drug effect, which also corresponds to the descriptive reduction in this frequency range in Zhang et al. (2020). Despite the overall lower slow spindle power, we observed an increase in the coupling of fast spindles to slow oscillations during the first 2–3 h of sleep, which is well in line with earlier findings of increased spindle–SO coupling upon zolpidem administration (Niknazar et al. 2015; Zhang et al. 2020). Apart from an increase in spindle power, previous studies have also observed reductions in the theta frequency range with zolpidem, particularly during the first part of the night (Brunner et al. 1991; Zhang et al. 2020). This is in line with the observed reduction of theta power during the first 2–3 h of sleep in the present study. Interestingly, we also observed a stronger coupling of theta events

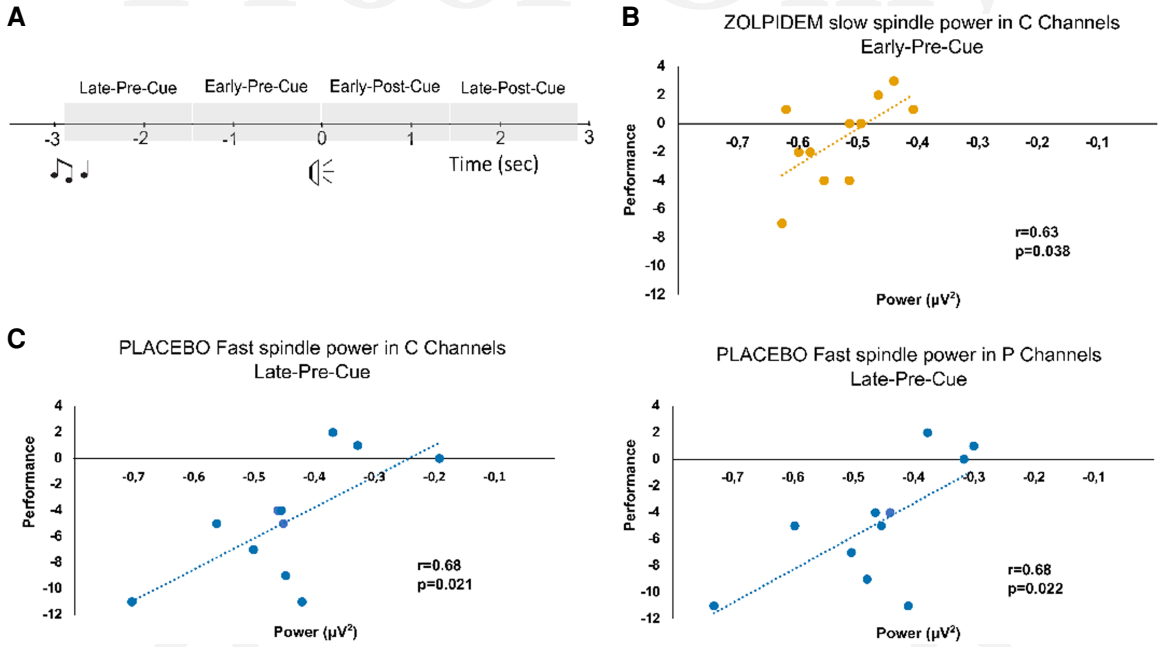

Figure 6. Associations between reminder-induced spindle responses and memory performance. (*A*) For exploratory correlation analyses, four time windows of interest were defined within the reactivation trials, corresponding to 1.4-sec intervals termed with regard to syllable (i.e., cue) onset: late-pre-cue (−2.8 to −1.4 sec), early-pre-cue (−1.4 to 0 sec), early-post-cue (0 to 1.4 sec), and late-post-cue (1.4 to 2.8 sec). (*B*) Slow spindle power in the early-pre-cue interval correlated with memory performance in the zolpidem group (for central electrodes, i.e., C channels), whereas fast spindle power in the late-pre-cue interval correlated with memory performance in the placebo group (for central and parietal electrodes, i.e., C and P channels). Please note that *P* values are reported uncorrected, and significant differences do not survive corrections for multiple comparisons.

to slow oscillations, particularly during the first 2–3 h of sleep. However, because neither the coupling measures nor the power reductions were associated with memory performance in the present study and some of the observed effects did not survive corrections for multiple comparisons, these findings should be interpreted with caution and should be scrutinized more systematically in future studies.

Examining the specific oscillatory responses upon the presentation of reminder cues during sleep, we found an elicited ERP accompanied by power increases in theta and fast spindle bands, which is well in line with previous studies on auditory targeted memory reactivation (Schreiner and Rasch 2015; Göldi et al. 2019; Schechtman et al. 2021). These results also replicate our previous findings with the same memory task and reactivation protocol (Forcato et al. 2020). Interestingly, although the responses to the syllable cue were equally expressed in both the zolpidem and placebo group, we observed a reduced response to the first part of the cue (i.e., the sound) in the slow spindle band following zolpidem administration. Moreover, slow spindle power in this cluster window was positively correlated with memory performance in the zolpidem group, although this correlation did not survive corrections for multiple comparisons. Nevertheless, this exploratory finding may tentatively suggest that even though zolpidem leads to an overall reduced response in the slow spindle band, participants who had the least reduction in slow spindle power may show a better memory retention. In the literature on sleep and memory, the functional role of slow spindles is less clear than that of fast spindles. Some studies have shown that slow and fast spindles differ in phase in their synchrony with the slow oscillation cycle, with fast spindles preferentially occurring in the depolarizing up-state of the slow oscillation and slow spindles being mainly linked to the transition from the up-state to the hyperpolarizing down-state (Mölle et al. 2011). Both types of spindles also differ in their generating mechanisms, with slow spindles being more closely linked to Na^+-channel dependent cortical excitability and fast spindles relying more on corticothalamic input to thalamic spindle generators (Ayoub et al. 2013). These findings suggest that the top-down control by slow oscillations differentially affects slow and fast spindles, with slow spindles predominantly reflecting interactions within corticocortical networks, whereas fast spindles are more closely linked to thalamocortical loops (Doran 2003; Mölle et al. 2011). Slow and fast spindles may synergistically interact to provide optimal memory consolidation. Fast spindles possibly represent a mechanism to relay memory-related information from the hippocampus to the neocortex via the nesting of hippocampal sharp-wave/ripples into fast spindle troughs (Siapas and Wilson 1998; Marshall and Born 2007), whereas subsequent slow spindles may be related to a cross-linking of the relayed information within and between different cortical networks. This functional differentiation may be further affected by zolpidem. Whereas we found associations between memory performance and slow spindle responses in the zolpidem group, the placebo group showed associations with fast spindle responses, although again, this association did not survive corrections for multiple comparisons. It could be speculated that zolpidem shifts the reliance of memory reactivation to a larger degree to direct corticocortical interactions (as supported by slow spindles), whereas under natural conditions the information flow between hippocampal and neocortical regions (as supported by fast spindles) may be essential. Yet, considering the weak effects in the present study, this interpretation should certainly be treated with caution. An interesting observation in relation to this interpretation is the finding that the reduction in slow spindle power following zolpidem administration occurred in response to the sound (i.e., shortly before the onset of the syllable). Based on our previous findings (Forcato et al. 2020), we would have expected an effect of zolpidem later during reminder presentation (i.e., in response to the syllable), and we can only speculate about possible explanations for

this effect. Recent studies have suggested that the effectiveness of externally triggered memory reactivation is determined by its temporal relationship with spontaneously occurring sleep spindles, with a refractory period after sleep spindles hindering the effective processing of external reactivation cues (Antony et al. 2018; Cairney et al. 2018; Antony et al. 2019). Based on these findings, it could be speculated that the reduced spindle response to the sound in the zolpidem group also reduced the refractoriness of spindles, thereby allowing for a more effective processing of the subsequent syllable cue. This possibility should be subject to further investigation.

One important limitation of the present study is the fact that we did not include a control condition without reactivation. Neither did we include a group without reactivation nor a within-subject comparison of reactivated and nonreactivated information. Thus, it could be argued that zolpidem simply increased memory consolidation during sleep, as has been previously shown, but did not specifically add to the targeted memory reactivation effect. However, based on extensive previous evidence on targeted memory reactivation, including our own previous work, we are reasonably confident that the targeted memory reactivation in our study worked effectively and already enhanced memory performance in the placebo group. First, previous evidence on targeted memory reactivation shows that declarative associative memory tasks quite consistently benefit from auditory reactivation protocols like the one applied in the present study, which was also supported by a recent meta-analysis (Hu et al. 2020; Klinzing and Diekelmann 2020). Second, our group has previously applied the exact same memory task and reactivation protocol and found convincing reactivation effects that were replicated three times, with a full night of sleep, 40 min of sleep, and a longer retention interval of 40 min sleep with additional 7 h wakefulness (Forcato et al. 2020). Third, the findings of the time–frequency analysis of the present study show very similar evoked responses to the reminders as in our previous experiments, indicating that the reactivation protocol worked well in the present study, similar to our previous three replications (Forcato et al. 2020). Thus, we believe that the reactivation protocol enhanced memory performance in the placebo group of the present study, indicating that zolpidem added to this enhancement beyond the targeted memory reactivation effect. However, without the appropriate control condition, we cannot be certain of this. We suggest that the present findings should be taken as first tentative evidence that zolpidem might increase the effects of targeted memory reactivation, but this needs to be replicated in future studies with appropriate control conditions. Such control conditions would also help to quantify the extent of the TMR effect and the additional zolpidem effect, which we were not able to do here.

Another limitation of the present study is the fact that zolpidem and placebo were compared in a between-subjects design, which might have introduced interindividual group differences. Particularly, for the observed differences in sleep parameters, it could be argued that these differences are attributable to interindividual differences between groups and not to an active drug effect. We found group differences particularly for spindle power, and it is well known that several spindle measures reflect trait-like differences between individuals (De Gennaro et al. 2005) and these differences are also related to cognitive abilities (Schabus et al. 2006; Fogel et al. 2007; Fang et al. 2017). Although we cannot fully exclude this possibility, our pattern of results speaks against it. Particularly for the time course of power differences across the night as well as for the coupling analyses, we found differences in spindle and theta power as well as in spindle–SO and theta–SO coupling that were closely related to the half-life of zolpidem; that is, group differences were selectively observed during the period of the maximum drug effect and were no longer evident during the second half of the night when the effect of zolpidem was expected to be much weaker. If the observed differences were attributable to trait factors, we would have expected general differences in sleep measures across the entire night and not only during the active drug phase. Nevertheless, interindividual differences may have weakened the effects observed in the present study and therefore future studies should apply within-subject designs to rule out any potential confounds of general group differences.

Further limitations include the overall small sample size (n = 11 per group) and potential selection biases due to the fact that we only included male subjects, leaving open the question whether similar findings would be observed with female participants. These issues need to be systematically addressed by further research.

Materials and Methods

Participants

Twenty-nine male participants were enrolled in the study and received financial compensation for their participation. All participants gave written informed consent and the study was approved by the local ethics committee of the Medical Faculty of the University of Tübingen. All participants were nonsmokers; did not suffer from any sleep disorder; did not have a history of any neurological, psychiatric, or endocrine disorder; did not take any medication; and reported to be in good health at the time of the experiments. None of the participants had done shift work for at least 6 wk prior to the experiments. From the 29 participants, seven were excluded from the final analyses because of an incorrect audio recording (two subjects), an incomplete reactivation session (one subject), or a misunderstanding in the instructions (one subject), because they did not reach the training session's learning criterion of 40% correct responses (one subject), or because it took them >90 min to fall asleep (two subjects); thus, there were 22 remaining subjects to be included in the analyses (mean age 25.0 ± 0.5). Sample size was determined based on power analyses with expected effect sizes estimated from previous experiments from our group using the same memory task (Forcato et al. 2020). Specifically, we assumed a large effect size of d = 1.1 (based on the three comparisons between the "incomplete reminder" and "no reminder" groups from Study 2 in Forcato et al. 2020, i.e., Exp. 2, 3, and 4), an α of 0.05, and power of 0.8, which resulted in a sample size of n = 11 per group.

Design and procedure

Subjects participated either in the zolpidem group (n = 11) or in the placebo group (n = 11) (Fig. 1A). In both groups, subjects spent an adaptation night in the sleep lab before the experimental night to become accustomed to the environment and electrode placement for sleep recordings. For experimental nights, participants arrived at the laboratory at 21:00 and were prepared for polysomnographic recordings in the same bedroom as for the adaptation night. At 22:00, they filled out the Stanford Sleepiness Scale (SSS) before the training session of the memory task started at about 22:05. Before going to bed at 23:00, participants were administered a pill that contained either 10 mg zolpidem or placebo in a double-blind fashion. After lights out, the experimenter monitored the sleep recording online and started the reactivation session after 10 min of stable SWS. After 8 h of sleep, participants were awakened and electrodes were removed. At least 30 min after awakening, to allow for the dissipation of sleep inertia, subjects filled out the SSS again and were then trained on the interference learning task. After another 30 min break, participants completed the SSS a third time and performed the testing session, in which the retrieval of the original memory task was assessed. Finally, participants completed the "heard/not-heard" task.

Memory task

The memory task consisted of 30 associations between semantically related sounds and German words (e.g., the sound of a storm associated with the word LAWINE [avalanche]). Each sound had a duration between 2855 and 2940 msec (on average 2900 msec). All words had three syllables and were prerecorded by a female voice.

Training session

Each trial started with the presentation of the sound for ~2900 msec. The sound then continued being played in the background while the associated word appeared written on the screen and spoken aloud once via headphones for 1500 msec (Fig. 1B). Thus, the sound was continuously repeated during the presentation of the associated word for the entire duration of the reminder. After a 4000-msec break, the next association was presented. After all 30 associations had been presented once, subjects performed a cued-recall test to get an immediate measure of learning performance. The cued-recall test consisted of the presentation of each association again as follows: the sound was presented for ~2900 msec alone, then the sound was repeated continuously in the background while the first syllable of the associated word was presented spoken aloud for 1500 msec. Subjects were asked to say the complete word out loud, for which they had a time limit of 5000 msec. Ater that, and independently of the subjects' answer, feedback was presented in the form of the sound and the correct word both written on the screen and aloud via headphones for 1500 msec. Subjects that did not reach 40% correct responses (12 correct answers) were excluded from the analysis.

Reactivation session

Participants were asked to put on in-ear headphones just before going to sleep, and they confirmed that they were able to hear the white noise (43 dB), which was presented from the moment they went to bed until the reactivation session finished. The material used for TMR consisted of incomplete reminders (sound + first syllable of the associated word), similar to the material used for the cued recall test in the training session. For each reminder, the sound was presented alone for 2900 msec, and then the sound was repeated continuously in the background while the first syllable of the associated word was presented spoken aloud for 1500 msec (45 dB). The next reminder was presented after of a break of 7000 msec until all of the 30 reminders were presented once. Presentation of the reminders started upon the detection of 10 min of stable SWS in the first SWS period. The entire reactivation procedure took about 5 min and 45 sec. Reactivation was paused whenever signs of arousal or changes in sleep stage were detected and resumed as soon as stable SWS was reached again.

Interference task

The interference task consisted of the same sounds from the training session but associated with new words. These words were also semantically related to the sounds and likewise consisted of three syllables, but with a different first syllable than the words of the original task. The training procedure was identical to the training session of the original task. Note that we did not aim to compare the interference task with a "no interference" condition, but this task was simply introduced to increase the likelihood of detecting sleep benefits at testing by making memory retrieval more challenging through prior interference learning. This paradigm has previously been shown to be well suited for unmasking subtle sleep effects on memory (Ellenbogen et al. 2006, 2009).

Testing session

For each of the 30 sound–word associations, the sound was played for 5500 msec and subjects were asked to say the associated word of the original memory task aloud. After a break of 4000 msec, the procedure continued until all 30 associations were presented.

Control tasks

Subjects rated their subjective sleepiness on the SSS ranging from 1 ("feeling active, vital, alert, or wide awake") to 7 ("no longer fighting sleep, sleep onset soon; having dream-like thoughts"). In the "heard/not-heard" task, participants were asked whether they had heard any sounds or words while they were sleeping. Additionally, all sounds plus first syllables were presented again and subjects had to indicate if they had received those stimuli while they were sleeping. Please note that no additional new sounds were presented in this task, precluding any assessment of false alarm rates and, thus, overall recognition performance. Measures of this task should simply be taken to assess whether there were any differences in the recognition of presented sounds between experimental groups.

Sleep recordings

Standard polysomnography including electroencephalographic (EEG), electromyographic (EMG), and electrooculographic (EOG) recordings was obtained with BrainAmp amplifiers (Brain Products). EEG was recorded from six scalp electrodes (F3, F4, C3, C4, P3, and P4 according to the International 10–20 System) and two electrodes on the left and right mastoids served as a combined reference. Data were recorded at a sampling rate of 200 Hz and bandpass-filtered between 0.16 and 35 Hz. Polysomnographic recordings were scored offline as wake, stage 1, stage 2, stages 3, and 4 (SWS), and REM sleep according to standard criteria (Rechtschaffen and Kales 1968). To test for associations between relevant sleep stages and memory performance, correlations were performed for stage 2, SWS and REM sleep.

Sleep data analysis

Sleep EEG data were analyzed using SpiSOP (https://www.spisop.org, RRID:SCR_015673) or custom-made codes in MATLAB 2013b (Mathworks).

Spindle and slow oscillation analyses

Slow oscillations, slow spindles, and fast spindles were detected with SpiSOP (https://www.spisop.org, RRID:SCR_015673), which is based on code of FieldTrip77 (http://fieldtriptoolbox.org, RRID:SCR_004849) in MATLAB 2013b (Mathworks) (Klinzing et al. 2016; Rudzik et al. 2018; Cha et al. 2020). The power spectrum of NREM sleep was used to visually select the peak of slow and fast spindles individually for each participant. Mean individual peak frequencies did not differ between the zolpidem and placebo groups for slow spindles (11.05 ± 0.32 vs. 10.73 ± 0.23, $P = 0.41$) and fast spindles (13.31 ± 0.15 vs. 13.39 0.14, $P = 0.70$). For the slow and fast spindles, the signal was filtered using a 2-Hz frequency band centered around the peak. After that, the root mean square of the signal, followed by a moving average of 0.2 sec, was applied to obtain a smoothed RMS signal. The envelope of this smoothed signal was then thresholded (1.5 s.d. away from the mean) to obtain the beginning and end of the spindles. For the slow oscillation detection, the signal was filtered between 0.3 to 3.5 Hz. Then, a slow oscillation was detected if the interval between two consecutive positive-to-negative zero crossings was between 0.8 and 2 sec (corresponding to the frequency of SOs between 0.5 and 1.25 Hz). The count and density of slow oscillations, slow spindles, and fast spindles was then determined for the six electrode positions and values from the left and right hemisphere were averaged into frontal (F), central (C), and parietal (P). This was done separately for stage 2 and SWS.

Power spectral analyses

Power spectra were also calculated using SpiSOP (https://www.spisop.org, RRID:SCR_015673), which is based on code of FieldTrip77 (http://fieldtriptoolbox.org, RRID:SCR_004849) in MATLAB 2013b (Mathworks). Sleep epochs of stage 2 and SWS were divided into consecutive 5-sec blocks with an overlap of 0.9 sec. The epochs were tapered by a single Hanning window

before applying fast Fourier transformation. Power spectra were then averaged across all blocks (Welch's method) and values for electrodes from the left and right hemisphere were averaged into frontal (F), central (C), and parietal (P). Mean power was calculated for the frequency bands of interest: slow oscillations (0.5–1 Hz), delta (1–4 Hz), theta (4–8 Hz), slow spindles (9–12 Hz), fast spindles (12–15 Hz), and the total spindle range (i.e., sigma, 9–15 Hz), separately for stage 2 and SWS. Note that power values for spindles and slow oscillations were calculated independently of the individually detected spindles and slow oscillations.

Time course of spindle and theta power across the night

Power values for the slow spindle, fast spindle, and theta frequency bands were calculated for each SWS sleep epoch (i.e., 30 sec of EEG recording scored as stage 3 or stage 4). Power values obtained for each epoch were averaged between subjects for zolpidem and placebo groups (e.g., all first SWS epochs from the 11 subjects in the zolpidem group were averaged). In an additional analysis, power values were averaged for each sleep cycle, with a sleep cycle being considered as a period between stable REM periods, which resulted in four sleep cycles across the night.

Coupling of slow oscillations with spindles and theta

To determine the degree of coupling between SOs and other frequency bands of interest (i.e., slow spindles, fast spindles, and theta), we calculated the modulation index (MI) following Canolty et al. (2006), separately for frontal, central, and parietal electrodes (averaged across left and right hemispheres). We first detected the SO events and extracted segments of ± 2.5 sec (with $t = 0$ referring to the negative peak of the SO). For each of these segments, we created a complex signal by combining the phase of the SO with the amplitude of either slow spindles, fast spindles, or theta (obtained through the Hilbert transform), reflecting a metric of the coupling between the two respective events (MI_raw). This metric was then normalized by calculating a set of surrogate means by introducing random offsets between the SO amplitude and the phase of the other frequencies. The normalized (or *z*-scored) MI was then calculated as $MI = (MI\_raw - \mu)/\sigma$, where μ and σ are the mean and standard deviation of the surrogate means. The MI was calculated separately for total NREM sleep, NREM sleep during the first 2.5 h of sleep (representing roughly the half-life of zolpidem, termed "half-life"), and NREM sleep during the rest of the night—that is, after the first 2.5 h of sleep (representing the time after the half-life of zolpidem, termed "post-half-life").

To visualize the angle-phase coupling, peri-event histograms for each frequency band and SO were calculated (Fig. 4B,C). They represent the amount of events (in %) that occurred at specific phases of each of the SO, with $t = 0$ representing the negative peak (down-state) of the SOs detected. For instance, a peak of 20 in $t = 0$ in the slow spindle/SO histogram would mean that a slow spindle was found in the negative peak of 20% of the SOs detected.

Time–frequency analyses

For each reminder, EEG data were cut into 12-sec trials (from −7 to 5 sec, with $t = 0$ sec representing the syllable onset). Trials containing artefacts in any of the channels were removed using automatic and visual rejection. After this process, a total of six trials were rejected from the whole data set. Time-frequency analyses were performed on the average of C channel electrodes, separately for zolpidem and placebo groups. The power was calculated relative to a 1-sec baseline from −4 and −3 sec (i.e., right before sound onset). This processing resulted in the relative power of each frequency at each time point. To evaluate the subjects' response to the reactivations, the average event-related response (ERP) was calculated for each group. To test for differences in the time–frequency representations between zolpidem and placebo, data were masked by cluster permutation statistics contrasting comparable time windows between both groups. Specifically, we used sample-level two-tailed independent-samples *t*-tests followed by a nonparametric cluster-permutation procedure to correct for multiple comparisons (1000 permutations), as implemented in the open-source toolbox FieldTrip (Forcato et al. 2020).

To perform correlations between memory performance and responses in the reactivation period, the trials were cut into shorter time windows of 1.4 sec termed with regard to syllable (cue) onset: late-pre-cue (−2.8 to −1.4 sec), early-pre-cue (−1.4 to 0 sec), early-post-cue (0 to 1.4 sec), and late-post-cue (1.4 to 2.8 sec) (Fig. 6A). Power values were calculated for these time windows in combined F, C, and P channels for the following frequency bands: slow spindles (9–12 Hz), fast spindles (12–16 Hz), and theta (4–8 Hz).

Statistical analysis

All statistical analyses for behavioral results and sleep parameters were performed with SPSS (version 25.0). Memory change (i.e., the number of correct responses at testing minus the number of correct responses at training) was calculated as memory performance measure. Comparisons between zolpidem and placebo were done with independent sample *t*-tests for memory performance, learning measures, control tasks, sleep stages, and the different sleep parameters such as spindle and SO count and density, power in different frequency bands, and spindle–SO and theta–SO coupling. Statistical comparisons for the time course of spindle and theta power across the night was also done with independent sample *t*-tests for each SWS epoch. Spindle–SO and theta–SO coupling for the half-life and post-half-life periods was analyzed with ANOVAs with the between-subjects factor zolpidem/placebo and the within-subjects factor half-life/post-half-life. Correlations between memory performance and any of the sleep and EEG measures were conducted with bivariate Pearson correlation coefficients. For all exploratory analyses regarding sleep and EEG measures, Bonferroni correction for multiple comparisons was applied. In some cases, both corrected and uncorrected results are reported (indicated at the respective places in the Results section). A value of $P < 0.05$ was considered significant.

Acknowledgments

This work was funded by a collaborative grant from the Deutsche Forschungsgemeinschaft (DFG) and the Consejo Nacional de Investigaciones Cientificas y Técnicas/Ministerio de Ciencia, Tecnologia e Innovación de la Nación (CONICET/MINCYT) to S. D. (DI 1866/2-1) and C.F. (Resolución D. No 4427) and a doctoral research fellowship from the Deutscher Akademischer Austauschdienst (DAAD) to J.C.

References

Antony JW, Piloto L, Wang M, Pacheco P, Norman KA, Paller KA. 2018. Sleep spindle refractoriness segregates periods of memory reactivation. *Curr Biol* **28:** 1736–1743.e4. doi:10.1016/j.cub.2018.04.020

Antony JW, Schönauer M, Staresina BP, Cairney SA. 2019. Sleep spindles and memory reprocessing. *Trends Neurosci* **42:** 1–3. doi:10.1016/j.tins.2018.09.012

Ayoub A, Aumann D, Hörschelmann A, Kouchekmanesch A, Paul P, Born J, Marshall L. 2013. Differential effects on fast and slow spindle activity, and the sleep slow oscillation in humans with carbamazepine and flunarizine to antagonize voltage-dependent Na^+ and Ca^{2+} channel activity. *Sleep* **36:** 905–911. doi:10.5665/sleep.2722

Bar E, Marmelshtein A, Arzi A, Perl O, Livne E, Hizmi E, Paz R, Sobel N, Dudai Y, Nir Y. 2020. Local targeted memory reactivation in human sleep. *Curr Biol* **30:** 1435–1446.e5. doi:10.1016/j.cub.2020.01.091

Brunner DP, Dijk DJ, Münch M, Borbély AA. 1991. Effect of zolpidem on sleep and sleep EEG spectra in healthy young men. *Psychopharmacology (Berl)* **104:** 1–5. doi:10.1007/BF02244546

Cairney SA, Guttesen AV, El Marj N, Staresina BP. 2018. Memory consolidation is linked to spindle-mediated information processing during sleep. *Curr Biol* **28:** 948–954. doi:10.1016/j.cub.2018.01.087

Canolty RT, Edwards E, Dalal SS, Soltani M, Nagarajan SS, Kirsch HE, Berger MS, Barbaro NM, Knight RT. 2006. High gamma power is phase-locked to theta oscillations in human neocortex. *Science* **313:** 1626–1628. doi:10.1126/science.1128115

Cha KS, Kim T-J, Jun J-S, Byun J-I, Sunwoo J-S, Shin J-W, Kim KH, Lee SK, Jung K-Y. 2020. Impaired slow oscillation, sleep spindle, and slow

oscillation–spindle coordination in patients with idiopathic restless legs syndrome. *Sleep Med* **66:** 139–147. doi:10.1016/j.sleep.2019.09.021
Chapouthier G, Venault P. 2002. GABA-A receptor complex and memory processes. *Curr Top Med Chem* **2:** 841–851. doi:10.2174/1568026023393552
Collinson N, Kuenzi FM, Jarolimek W, Maubach KA, Cothliff R, Sur C, Smith A, Otu FM, Howell O, Atack JR, et al. 2002. Enhanced learning and memory and altered GABAergic synaptic transmission in mice lacking the 5 subunit of the GABA A receptor. *J Neurosci* **22:** 5572–5580. doi:10.1523/JNEUROSCI.22-13-05572.2002
Comeras LB, Hörmer N, Mohan Bethuraj P, Tasan RO. 2021. NPY released from GABA neurons of the dentate gyrus specially reduces contextual fear without affecting cued or trace fear. *Front Synaptic Neurosci* **13:** 635726. doi:10.3389/fnsyn.2021.635726
Cox R, Hofman WF, de Boer M, Talamini LM. 2014. Local sleep spindle modulations in relation to specific memory cues. *Neuroimage* **99:** 103–110. doi:10.1016/j.neuroimage.2014.05.028
Cox R, Rüber T, Staresina BP, Fell J. 2019. Heterogeneous profiles of coupled sleep oscillations in human hippocampus. *Neuroimage* **202:** 116178. doi:10.1016/j.neuroimage.2019.116178
De Gennaro L, Ferrara M, Vecchio F, Curcio G, Bertini M. 2005. An electroencephalographic fingerprint of human sleep. *Neuroimage* **26:** 114–122. doi:10.1016/j.neuroimage.2005.01.020
Diekelmann S, Born J. 2010. The memory function of sleep. *Nat Rev Neurosci* **11:** 114–126. doi:10.1038/nrn2762
Diekelmann S, Büchel C, Born J, Rasch B. 2011. Labile or stable: opposing consequences for memory when reactivated during waking and sleep. *Nat Neurosci* **14:** 381–386. doi:10.1038/nn.2744
Dijk DJ, James LM, Peters S, Walsh JK, Deacon S. 2010. Sex differences and the effect of gaboxadol and zolpidem on EEG power spectra in NREM and REM sleep. *J Psychopharmacol* **24:** 1613–1618. doi:10.1177/0269881109105788
Doran S. 2003. The dynamic topography of individual sleep spindles. *Sleep Res Online* **5:** 133–139.
Ellenbogen JM, Hulbert JC, Stickgold R, Dinges DF, Thompson-Schill SL. 2006. Interfering with theories of sleep and memory: sleep, declarative memory, and associative interference. *Curr Biol* **16:** 1290–1294. doi:10.1016/j.cub.2006.05.024
Ellenbogen JM, Hulbert JC, Jiang Y, Stickgold R. 2009. The sleeping brain's influence on verbal memory: boosting resistance to interference. *PLoS ONE* **4:** e4117. doi:10.1371/journal.pone.0004117
Fang Z, Sergeeva V, Ray L, Viczko J, Owen A, Fogel S. 2017. Sleep spindles and intellectual ability: epiphenomenon or directly related? *J Cogn Neurosci* **29:** 167–182. doi:10.1162/jocn_a_01034
Feld GB, Wilhelm I, Ma Y, Groch S, Binkofski F, Mölle M, Born J. 2013. Slow wave sleep induced by gaba agonist tiagabine fails to benefit memory consolidation. *Sleep* **36:** 1317–1326. doi:10.5665/sleep.2954
Fogel SM, Nader R, Cote KA, Smith CT. 2007. Sleep spindles and learning potential. *Behav Neurosci* **121:** 1–10. doi:10.1037/0735-7044.121.1.1
Forcato C, Klinzing JG, Carbone J, Radloff M, Weber FD, Born J, Diekelmann S. 2020. Reactivation during sleep with incomplete reminder cues rather than complete ones stabilizes long-term memory in humans. *Commun Biol* **3:** 1–13. doi:10.1038/s42003-020-01457-4
Göldi M, Anna E, Van Poppel M, Rasch B, Schreiner T. 2019. Increased neuronal signatures of targeted memory reactivation during slow-wave up states. *Sci Rep* **9:** 2715. doi:10.1038/s41598-019-39178-2
Gonzalez CE, Mak-McCully RA, Rosen BQ, Cash SS, Chauvel PY, Bastuji H, Rey M, Halgren E. 2018. Theta bursts precede, and spindles follow, cortical and thalamic downstates in human NREM sleep. *J Neurosci* **38:** 9989–10001. doi:10.1523/JNEUROSCI.0476-18.2018
Heaney CF, Kinney JW. 2016. Role of $GABA_B$ receptors in learning and memory and neurological disorders. *Neurosci Biobehav Rev* **63:** 1–28. doi:10.1016/j.neubiorev.2016.01.007
Helfrich RF, Mander BA, Jagust WJ, Knight RT, Walker MP. 2018. Old brains come uncoupled in sleep: slow wave-spindle synchrony, brain atrophy, and forgetting. *Neuron* **97:** 221–230. doi:10.1016/j.neuron.2017.11.020
Hu X, Cheng LY, Paller KA. 2020. Promoting memory consolidation during sleep : a meta-analysis of targeted memory reactivation. *Psychol Bull* **146:** 218–244. doi:10.1037/bul0000223
Izquierdo I, Medina JH. 1991. $GABA_A$ receptor modulation of memory: the role of endogenous benzodiazepines. *Trends Pharmacol Sci* **12:** 260–265. doi:10.1016/0165-6147(91)90567-C
Kaestner EJ, Wixted JT, Mednick SC. 2013. Pharmacologically increasing sleep spindles enhances recognition for negative and high-arousal memories. *J Cogn Neurosci* **25:** 1597–1610. doi:10.1162/jocn_a_00433
Kanno O, Sasaki T, Watanabe H, Takazawa S, Nakagome K, Nakajima T, Ichikawa I, Akaho R, Suzuki M. 2000. Comparison of the effects of zolpidem and triazolam on nocturnal sleep and sleep latency in the morning: a cross-over study in healthy young volunteers. *Prog Neuro Psychopharmacol Biol Psychiatry* **24:** 897–910. doi:10.1016/S0278-5846(00)00117-2
Klinzing JG, Diekelmann S. 2020. Chapter 31: Cued memory reactivation: a tool to manipulate memory consolidation during sleep. In *Handbook of sleep research* (ed. Dringenberg BN), Vol. 30, pp. 471–488, Elsevier. **Q3**
Klinzing JG, Mölle M, Weber F, Supp G, Hipp JF, Engel AK, Born J. 2016. Spindle activity phase-locked to sleep slow oscillations. *Neuroimage* **134:** 607–616. doi:10.1016/j.neuroimage.2016.04.031
Klinzing JG, Niethard N, Born J. 2019. Mechanisms of systems memory consolidation during sleep. *Nat Neurosci* **22:** 1598–1610. doi:10.1038/s41593-019-0467-3
Krugliakova E, Volk C, Jaramillo V, Sousouri G, Huber R. 2020. Changes in cross-frequency coupling following closed-loop auditory stimulation in non–rapid eye movement sleep. *Sci Rep* **10:** 10628. doi:10.1038/s41598-020-67392-w
Lancel M. 1999. Role of GABA(A) receptors in the regulation of sleep: initial sleep responses to peripherally administered modulators and agonists. *Sleep* **22:** 33–42. doi:10.1093/sleep/22.1.33
Lemmer B. 2007. The sleep–wake cycle and sleeping pills. *Physiol Behav* **90:** 285–293. doi:10.1016/j.physbeh.2006.09.006
Lewis PA, Bendor D. 2019. How targeted memory reactivation promotes the selective strengthening of memories in sleep. *Curr Biol* **29:** R906–R912. doi:10.1016/j.cub.2019.08.019
Lundahl J, Deacon S, Maurice D, Staner L. 2012. EEG spectral power density profiles during NREM sleep for gaboxadol and zolpidem in patients with primary insomnia. *J Psychopharmacol* **26:** 1081–1087. doi:10.1177/0269881111424457
Marshall L, Born J. 2007. The contribution of sleep to hippocampus-dependent memory consolidation. *Trends Cogn Sci* **11:** 442–450. doi:10.1016/j.tics.2007.09.001
Mednick SC, Cai DJ, Shuman T, Anagnostaras S, Wixted JT. 2011. An opportunistic theory of cellular and systems consolidation. *Trends Neurosci* **34:** 504–514. doi:10.1016/j.tins.2011.06.003
Mednick SC, McDevitt EA, Walsh JK, Wamsley E, Paulus M, Kanady JC, Drummond SPA. 2013. The critical role of sleep spindles in hippocampal-dependent memory: a pharmacology study. *J Neurosci* **33:** 4494–4504. doi:10.1523/JNEUROSCI.3127-12.2013
Mölle M, Eschenko O, Gais S, Sara SJ, Born J. 2009. The influence of learning on sleep slow oscillations and associated spindles and ripples in humans and rats. *Eur J Neurosci* **29:** 1071–1081. doi:10.1111/j.1460-9568.2009.06654.x
Mölle M, Bergmann TO, Marshall L, Born J. 2011. Fast and slow spindles during the sleep slow oscillation: disparate coalescence and engagement in memory processing. *Sleep* **34:** 1411–1421. doi:10.5665/SLEEP.1290
Ngo HV, Fell J, Staresina B. 2020. Sleep spindles mediate hippocampal-neocortical coupling during long-duration ripples. *Elife* **9:** e57011. doi:10.7554/eLife.57011
Niknazar M, Krishnan GP, Bazhenov M, Mednick SC. 2015. Coupling of thalamocortical sleep oscillations are important for memory consolidation in humans. *PLoS One* **10:** e0144720. doi:10.1371/journal.pone.0144720
Oudiette D, Paller KA. 2013. Upgrading the sleeping brain with targeted memory reactivation. *Trends Cogn Sci* **17:** 142–149. doi:10.1016/j.tics.2013.01.006
Peyrache A, Seibt J. 2020. A mechanism for learning with sleep spindles. *Philos Trans R Soc B Biol Sci* **375:** 20190230. doi:10.1098/rstb.2019.0230
Rasch B, Born J. 2007. Maintaining memories by reactivation. *Curr Opin Neurobiol* **17:** 698–703. doi:10.1016/j.conb.2007.11.007
Rechtschaffen A, Kales A. 1968. *A manual of standardized terminology, technique and scoring system for sleep stages of human sleep*. Brain Information Service, Brain Information Institute, UCLA, Los Angeles, CA.
Rihm JS, Diekelmann S, Born J, Rasch B. 2014. Reactivating memories during sleep by odors: odor specificity and associated changes in sleep oscillations. *J Cogn Neurosci* **26:** 1806–1818. doi:10.1162/jocn_a_00579
Rodríguez MLC, Campos J, Forcato C, Leiguarda R, Maldonado H, Molina VA, Pedreira ME. 2013. Enhancing a declarative memory in humans: the effect of clonazepam on reconsolidation. *Neuropharmacology* **64:** 432–442. doi:10.1016/j.neuropharm.2012.06.059
Rosanova M, Ulrich D. 2005. Pattern-specific associative long-term potentiation induced by a sleep spindle-related spike train. *J Neurosci* **25:** 9398–9405. doi:10.1523/JNEUROSCI.2149-05.2005
Rudoy JD, Voss JL, Westerberg CE, Paller KA. 2009. Strengthening individual memories by reactivating them during sleep. *Science* **326:** 1079. doi:10.1126/science.1179013
Rudzik F, Thiesse L, Pieren R, Wunderli JM, Brink M, Foraster M, Héritier H, Eze IC, Garbazza C, Vienneau D, et al. 2018. Sleep spindle characteristics and arousability from nighttime transportation noise exposure in healthy young and older individuals. *Sleep* **41:** zsy077. doi:10.1093/sleep/zsy077
Schabus M, Hödlmoser K, Gruber G, Sauter C, Anderer P, Klösch G, Parapatics S, Saletu B, Klimesch W, Zeitlhofer J. 2006. Sleep spindle–related activity in the human EEG and its relation to general cognitive

and learning abilities. *Eur J Neurosci* **23:** 1738–1746. doi:10.1111/j.1460-9568.2006.04694.x

Schechtman E, Antony JW, Lampe A, Wilson BJ, Norman KA, Paller KA. 2021. Multiple memories can be simultaneously reactivated during sleep as effectively as a single memory. *Commun Biol* **4:** 1–13. doi:10.1038/s42003-020-01512-0

Schreiner T, Rasch B. 2015. Boosting vocabulary learning by verbal cueing during sleep. *Cereb Cortex* **25:** 4169–4179. doi:10.1093/cercor/bhu139

Schreiner T, Lehmann M, Rasch B. 2015. Auditory feedback blocks memory benefits of cueing during sleep. *Nat Commun* **6:** 1–11. doi:10.1038/ncomms9729

Schreiner T, Doeller CF, Jensen O, Rasch B, Staudigl T. 2018. Theta phase–coordinated memory reactivation reoccurs in a slow-oscillatory rhythm during NREM sleep. *Cell Rep* **25:** 296–301. doi:10.1016/j.celrep.2018.09.037

Siapas AG, Wilson MA. 1998. Coordinated interactions between hippocampal ripples and cortical spindles during slow-wave sleep. *Neuron* **21:** 1123–1128. doi:10.1016/S0896-6273(00)80629-7

Staresina BP, Bergmann TO, Bonnefond M, Van Der Meij R, Jensen O, Deuker L, Elger CE, Axmacher N, Fell J. 2015. Hierarchical nesting of slow oscillations, spindles and ripples in the human hippocampus during sleep. *Nat Neurosci* **18:** 1679–1686. doi:10.1038/nn.4119

Uchimura N, Nakajima T, Hayash K, Nose I, Hashizume Y, Ohyama T, Habukawa M, Kotorii N, Kuwahara H, Maeda H. 2006. Effect of zolpidem on sleep architecture and its next-morning residual effect in insomniac patients: a randomized crossover comparative study with brotizolam. *Prog Neuro Psychopharmacol Biol Psychiatry* **30:** 22–29. doi:10.1016/j.pnpbp.2005.06.018

Ulrich D. 2016. Sleep spindles as facilitators of memory formation and learning. *Neural Plast* **2016:** 1–7. doi:10.1155/2016/1796715

Ulrich D, Lalanne T, Gassmann M, Bettler B. 2018. $GABA_B$ receptor subtypes differentially regulate thalamic spindle oscillations. *Neuropharmacology* **136:** 106–116. doi:10.1016/j.neuropharm.2017.10.033

Wilson MA, McNaughton BL. 1994. Reactivation of hippocampal ensemble memories during sleep. *Science* **265:** 676–679. doi:10.1126/science.8036517

Zhang J, Yetton B, Whitehurst LN, Naji M, Mednick SC. 2020. The effect of zolpidem on memory consolidation over a night of sleep. *Sleep* **43:** zsaa084.

Received March 31, 2021; accepted in revised form July 7, 2021.

LM052787Car

Queries

Julia Carbone et al.

Q1 Please provide postal codes for affiliations.

Q2 As outlined in our Instructions to Authors, it is the journal's style to set genes, alleles, and loci in italic, and proteins in Roman type. Please verify that all have been properly set throughout the manuscript.

Q3 Please provide publisher location for reference "Klinzing and Diekelmann 2020".