Memory Manipulation During Sleep: Fundamental Advances and Possibilities for Application

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Abstract Sleep is critically involved in cognitive functioning through content-specific information processing. Importantly, recent findings consistently show that these processes can be actively manipulated. For instance, by interfering with brain activity directly, or by presenting memory cues during sleep. This chapter will discuss recent advances in this field, considering basic research in both animals and human participants. Initial steps toward possible applications of sleep-related memory manipulations will also be discussed.

Keywords Targetted memory reactivation · Memory manipulation · Memory implantation

An increasing body of evidence suggests that sleep is not only essential for physical health, but also for a variety of mental functions. For instance, learning, memory consolidation and emotional coping all appear to benefit from sleep. The importance of sleep for mental function is not merely due to a general restorative effect of sleep. Rather, it seems to be related to brain-specific processes, whereby information acquired during the day is reprocessed and reorganised (Stickgold 2013). Pivotal contributions to our understanding of these processes, especially regarding the memory function of sleep, have come from studies using manipulations to directly influence these sleep-related processes. Some such manipulations have been aimed at neuronal activity, imposing artificial memories during sleep, some act on oscillatory population dynamics, boosting or interrupting particular EEG patterns, while still others have targeted the system at the sensory level, through presentation of memory cues during sleep. A recent development in this field

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N. Axmacher and B. Rasch (eds.), *Cognitive Neuroscience of Memory Consolidation*, Studies in Neuroscience, Psychology and Behavioral Economics, DOI 10.1007/978-3-319-45066-7_19

concerns the use of closed-loop stimulation techniques, to precisely target stimuli at particular neural activity patterns. Studies of this type have generated strong support for many aspects of the 'sleep consolidation hypothesis', providing causal evidence for the role of different neural activity patterns in memory consolidation. On a more practical level, the observation that memory content can be manipulated during sleep has invited the exciting idea that such manipulations might be applied to practical purposes. This chapter will start with a brief introduction to sleep-related memory processing and its neural underpinnings. The sections thereafter will present an overview of the literature on memory manipulation during sleep.

Sleep-Related Memory Processing

Sleep-related neural processes supporting memory have been most directly investigated in rodents, using tasks recruiting the hippocampus (typically spatial, episodic or working memory tasks). In such studies, beneficial effects of sleep on memory performance were shown to involve the reactivation of previously encoded neuronal representations in hippocampo-(neo)cortical circuits (Peyrache et al. 2009; Ji and Wilson 2007; Atherton et al. 2015). While this so-called 'replay' activity has been observed in both rapid eye movement (REM) sleep (Louie and Wilson 2001) and nonREM sleep, the importance of sleep for declarative memory has, thus far, mostly been attributed to nonREM sleep (Born et al. 2006) and its signature oscillatory events, slow oscillations (Marshall et al. 2006; Marshall et al. 2011), sleep spindles (Gais et al. 2002; Clemens et al. 2005; Clemens et al. 2006; Schmidt et al. 2006; van der Helm et al. 2011a; Cox et al. 2012; Kaestner et al. 2013; Mednick et al. 2013; Cox et al. 2014a) and hippocampal sharp-wave ripples (O'Neill et al. 2010).

The large-scale coordination of neural activity, necessary for the putative reactivation of distributed memory representations, is thought to be reflected in the spatiotemporal coupling of these brain rhythms (Buzsaki 1996; Clemens et al. 2007; Holleman and Battaglia 2015). Specifically, reactivation of memory representations appears to occur during sharp wave ripple events in the hippocampus (O'Neill et al. 2010). These sharp wave ripples are time locked to the depolarised phase of thalamocortical spindles, which themselves are grouped into the up-state of slow oscillations (Mölle et al. 2002; Cox et al. 2014b; Staresina et al. 2015) (see also chapter by Bergmann and Staresina). The temporal coupling of sharp wave-ripples and spindles is thought to reflect the combined reactivation of hippocampal and (neo)cortical components of memory representations during sleep. The slow oscillations, in turn, have an important role in the functional coupling of cortical networks (Cox et al. 2014b) (Fig. 1). The combined dynamics are thought to underlie the spatiotemporal orchestration of sleep-related memory reactivation and sleep-related information processing in general.

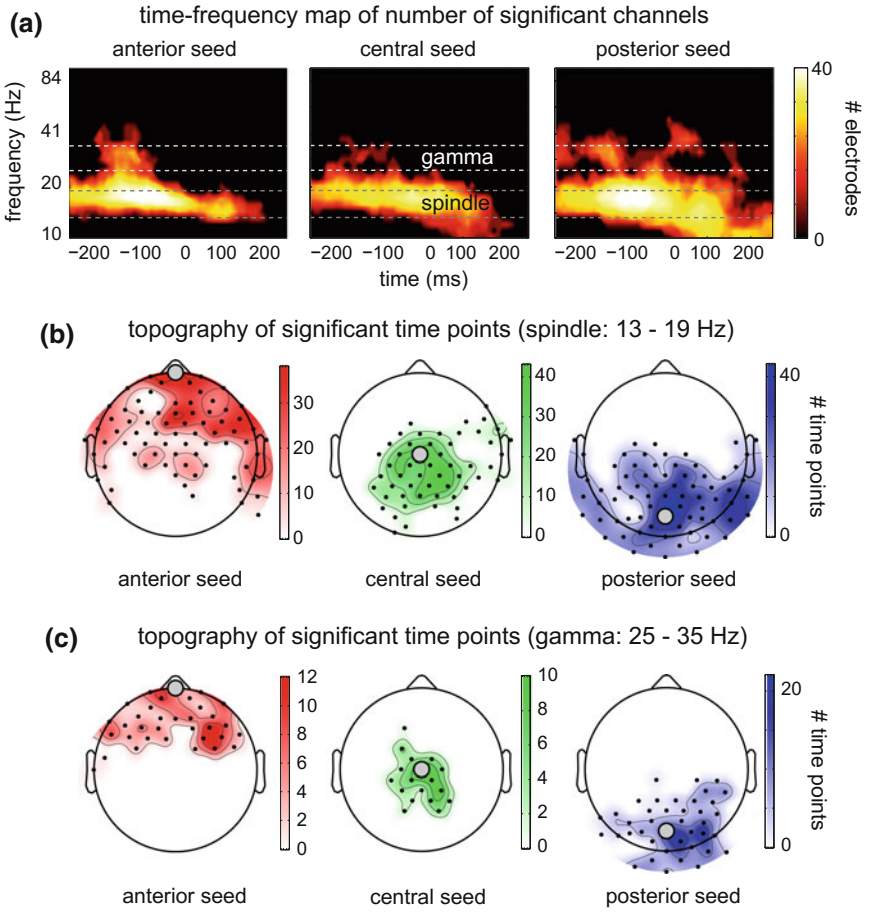


Fig. 1 High-density sleep-EEG (128 channels) was used to assess the spatiotemporal characteristics of oscillatory population dynamics during sleep. The figure shows the spatiotemporal extent of SO-phase effect on power. Three seed electrodes were used for SO detection. **a** Heat maps of clusters with significantly higher power in the up versus down state for an anterior (*left column*), central (*middle column*), and posterior seed electrode (*right column*). Indicated is the number of channels involved at every time–frequency point. Note the apparent presence of two distinct frequency ranges in these clusters, indicated between *dashed lines* and labelled "spindle" and "gamma." **b**, **c** Topographies of these frequency-specific effects (**b** spindles; **c** gamma) reveal that power modulations are highly localized. Channels closer to the seed electrode used for SO detection are involved in the significant cluster on more time points. Similarly, slow oscillations were found to modulate inter-site phase synchrony in the spindle range, as well as beta and gamma activity coupling to spindle phase (Cox et al. 2014b)

Manipulations of Oscillatory Sleep Patterns

Crucial insights regarding the neural underpinnings of sleep-related memory processing have come from studies involving precise manipulations of sleep physiology. The pertaining manipulations specifically enhance or suppress a particular neural activity pattern implicated in sleep-related memory processing. Importantly, studies using such methods have demonstrated the causal role of various sleep-related oscillations in memory consolidation.

A first such experiment applied transcranial, oscillating potentials (0.75 Hz) during early nocturnal non-REM sleep to boost slow oscillations in humans. The method was based on the more general finding that some endogenous brain rhythms can phase lock and resonate to external rhythms (Jefferys and Haas 1982; Hutcheon and Yarom 2000). Transcranial direct current stimulation (tDCS), indeed, led to increased slow oscillation power and slow spindle activity in short (1 min) intervals in between stimulations, and enhanced retention across sleep on a declarative memory task (Marshall et al. 2006; Marshall et al. 2004) (see chapter by Campos Beltran and Marshall). Later studies found that brief sound pulses applied upon detection of a slow oscillation negative half wave peak during nonREM sleep also led to boosting of slow oscillations and spindles, in a short period following the pulse (Ngo et al. 2013a, b) (Fig. 2). As in the study with tDCS, there was a concomitant declarative memory enhancement for material acquired prior to sleep. These studies support a causal involvement of slow oscillations in sleep-related memory processes.

Studies in rodents brought forth similar support for a causal role of spindles and sharp wave-ripples in memory processes (see also chapter by Maier and Kempter). For instance, pharmacological enhancement of spindle occurrence improved memory retention across sleep (Kaestner et al. 2013; Mednick et al. 2013) (see chapter by McDevitt, Krishnan, Bazhenov and Mednick), while interruption of sharp wave-ripples by closed-loop electrical stimulation interfered with rats' ability to recall spatial memory information (Girardeau et al. 2009; Ego-Stengel and Wilson 2010). Thus, a large body of physiological findings supporting the idea that memory consolidation is reflected in spatiotemporal sharp wave-ripple, spindle, slow oscillation coupling, was strengthened by evidence linking each of these three oscillatory phenomena causally to memory consolidation.

Targeted Memory Manipulation During Sleep

In the previous section, we saw that memory retention over sleep can be enhanced through manipulations of sleep physiology. A particularly exciting line of research concerns the possibility to target individual memories in the sleeping brain and manipulate these specifically. Indeed, according to recent studies, specific memories can be enhanced by presentation of memory cues during sleep. It is even possible to

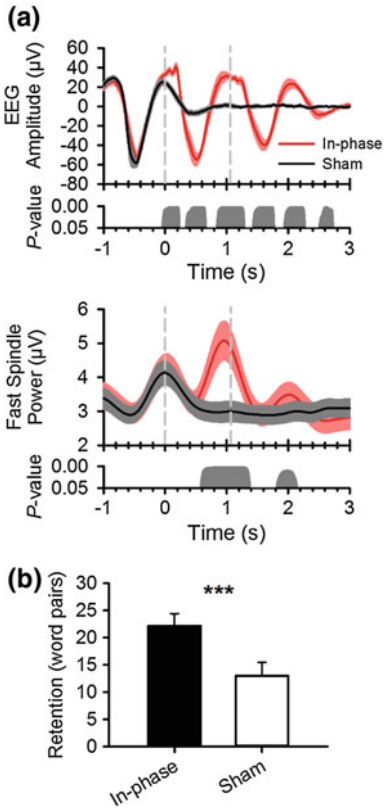


Fig. 2 Auditory stimulation in phase with slow oscillation up-states boosts sleep rhythms and promotes declarative memory. **a** During a so-called In-phase auditory stimulation condition (shown in *red*) participants were subjected to two brief clicks (50-ms *pink* noise) whenever online detection of a spontaneous slow oscillation occurred. These clicks were delivered such that they concurred with the next two up-coming slow oscillation up-states (*vertical grey lines*). This led to prolonging of the on-going slow oscillatory activity in comparison to a Sham control condition without stimulation (*black lines*, *upper panel*) and increased fast spindle power (12–15 Hz) phase-locked to the induced slow oscillation up-states (*lower panel*). *Bottom traces* indicate significant differences between conditions. **b** To assess effects on hippocampal memory consolidation subjects learned a declarative memory task of 120 paired associate words and performed cued recall before and after sleep. Retention (post-sleep minus pre-sleep recall performance) was distinctly higher during In-phase stimulation (*black bar*) than in the Sham condition (*empty bar*); *** $P < 0.001$

instil entirely new memories during sleep in the form of conditioned responses (Arzi et al. 2012). We might refer to such procedures, which target specific memory content during sleep, as 'targeted memory manipulations'.

The fact that sleep-related processing can be influenced by external cues may seem surprising, given the strongly reduced access of sensory input to thalamo-cortical circuits. However, despite this dampening, the sleeping brain

maintains a lingering receptiveness to external stimuli. Obviously, external stimuli can still lead to arousal and awakening, provided they are sufficiently strong. More interestingly, the extent to which external stimuli will be perceived during sleep depends on stimulus properties like familiarity and personal meaning. For instance, irrelevant, familiar stimuli, like household or street noises, may not disturb a sound sleeper, while even soft crying of a baby may quickly awaken the parents. This suggests that at least some level of sensory stimulus processing persists during sleep.

Evidence for this notion comes from studies evaluating sleeping subjects' brain responses (typically evoked potentials) in response to simple auditory or tactile stimuli (Bastuji and García-Larrea 1999). The general tenet from this body of work is that, while the sleeping brain responds differently from wakefulness, it retains some residual capacity for performing simple processing relating to stimulus salience, novelty and significance. Recent findings suggest that the sleeping brain can respond in a stimulus-specific manner even to more complex stimuli, and that, using this propensity of the sleeping brain, information processing can be manipulated at the content level. For instance, in an early experiment, object locations were remembered better if an odour, present during learning, was also present during subsequent slow wave sleep (SWS) (Rasch et al. 2007) (see also chapter by Shanahan and Gottfried). This effect was later shown to be odour specific (Rihm et al. 2014). That is, the memory enhancing effect of odour presentation was dependent on the prior association of the odour with the items.

The choice for odour cues in these early experiments was guided by the fact that odour stimuli have a low chance of leading to arousals during sleep compared to, for instance, auditory stimuli. Furthermore, the olfactory system bypasses the thalamus, connecting directly to olfactory cortex, which, in turn, projects heavily to the hippocampus (see chapter by Wilson, Kondrakiewicz and Barnes). Thus odour cues, compared to other sensory cues, may have a better chance of reaching the hippocampus and leading to cued reactivation of hippocampus-dependent memory traces. Nonetheless, several studies have show that auditory stimuli can also be used as memory cues during sleep (Rudoy et al. 2009; Antony et al. 2012; Oudiette et al. 2013; Schreiner and Rasch 2014; Schreiner et al. 2015) (see chapter by Schreiner, Lehmann and Rasch). From an experimental perspective, the advantage of auditory cues is that they can more easily be used to create multiple unique cue-to-memory pairings, allowing experiments in which individual memories are specifically reactivated.

This property was exploited in an experiment in which subjects learned object-location associations while hearing characteristic object sounds (Rudoy et al. 2009). Part of these sounds were presented again during nonREM sleep. Upon waking, subjects recalled the locations associated to the sleep cues more accurately than other locations for which no cues were provided (Fig. 3). In another study, auditory cues presented during SWS were used to enhance skill learning (Antony et al. 2012). Specifically, the production, using a keyboard, of one of two practiced melodies, was enhanced by presenting that melody during a nap. Finally, two recent studies showed that verbal cueing during sleep can boost vocabulary learning (Schreiner and Rasch 2014; Schreiner et al. 2015). In the latter experiments sleeping

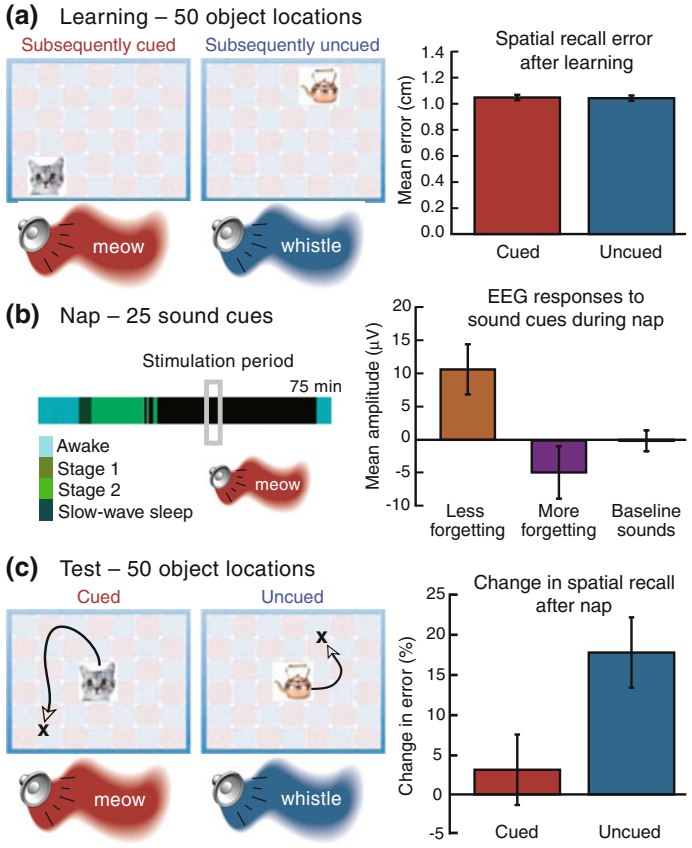


Fig. 3 Rudoy and colleagues (2009) manipulated memory processing during sleep using sounds. **a** In the initial stage of the experiment, individuals learned object-location associations on a grid while hearing the corresponding object sounds, using repeated presentations in a drop-out learning procedure. Accuracy was measured for each object as the distance between the remembered location and the original location, and accuracy at the conclusion of learning was matched for two sets of 25 objects, one set that was subsequently cued by the sounds during sleep and one that was not cued. **b** Sleep-staging data for a representative participant, showing the timing of the 3.5-min sequence of 25 sound cues that were presented at a very low level so as to not disturb ongoing sleep. Neural activity elicited by the sounds during sleep predicted later memory performance. EEG analyses showed that potentials at 400–800 ms after sound onset differed according to level of forgetting for corresponding object locations. **c** After the nap, individuals attempted to place each object in its correct location (*arrows* simulate motion of objects as individuals complete the task). Better spatial-location retention for cued compared to uncued objects was reflected by a smaller change in error, indicating that targeted memory reactivation through sounds presented during slow-wave sleep selectively improved spatial memory for corresponding objects

subjects were re-exposed, during nonREM sleep, to auditory presentations of foreign words learned prior to sleep. Upon waking, the correct translation of the foreign words to the mother tongue was more often recalled for sleep-cued respective to non-cued words (see Fig. 1 in the chapter of Schreiner, Lehman and Rasch).

The combined studies show that successful memory cueing during sleep can be achieved using different sensory cues, including olfactory and auditory ones. Such cueing can benefit memory performance over a variety of memory tasks, spanning the declarative and procedural memory domains. It might be noted that some of the above declarative memory studies administered memory cues in nonREM sleep (Rudoy et al. 2009; Schreiner and Rasch 2014; Schreiner et al. 2015), rather than SWS specifically (Rasch et al. 2007), which has more consistently been implicated in episodic/declarative memory consolidation (Stickgold 2013; Cox et al. 2012; Rasch and Born 2013; Sweegers and Talamini 2014). At the same time, the single study regarding skill learning (Antony et al. 2012) presented cues during SWS, which is not the sleep stage most strongly implicated in skill learning. It is as yet not clear whether cued memory reactivation during sleep benefits differently from cueing in different sleep stages and how this might depend on the type of cue and the type of memory task.

More in general, it might be noted that the size of the memory effects in these studies is typically modest and that memory benefits have not been observed in all studies. Interestingly, the larger part of studies showing benefits of sleep-cueing on post-sleep memory performance used declarative memory tasks with a clear semantic component to retrieval performance (Schreiner and Rasch 2014; Schreiner et al. 2015). This is also the case for studies showing enhanced memory retention following slow oscillation boosting (Marshall et al. 2006; Marshall et al. 2004; Ngo et al. 2013a, b). Examples of memory tasks used in these studies are paired associate tasks with semantically related associates and vocabulary learning tasks using related languages. On the other hand, studies using tasks relying more stringently on episodic memory did not always lead to behavioural memory improvement (Cox et al. 2014a; van Dongen et al. 2012). A study in our lab that used a strictly hippocampus-dependent word to location association task (similar to Cox et al. 2014a) and was optimized to detect possible effects on memory performance, also did not uncover such effects (Talamini and Cox, unpublished observations). While many experimental factors may underlie these differential outcomes, one possibility is that semantic memory effects may be induced through relatively local reactivations (e.g. within language networks for a verbal task), while episodic memory enhancements may require reactivation of larger scale networks, encompassing the hippocampus and widespread neocortical areas. Perhaps, effects based on relatively local processing are easier to achieve. Thus, while initial findings on targeted memory reactivation are highly exciting, many parameters determining the effectiveness of such procedures remain to be investigated.

Implanting New Memories

While the above studies regard the manipulation of existing memories during sleep, an equally intriguing question is whether novel information, processed during sleep, can leave lasting memory traces. A few older studies found either no evidence for

sleep-learning of verbal material (Emmons and Simon 1956; Wood et al. 1992) or had methodological difficulties (Fox and Robbin 1952). A recent study, involving slow oscillation-upstate-locked presentation of real world sounds during sleep, also didn't find traces of lasting memory formation (Cox et al. 2014c). However, it appears that a simpler form of learning, classical conditioning, can take place during sleep. In a study with human participants, pleasant and unpleasant odours were paired with different tones during sleep. During ensuing wake, subjects displayed sniff responses to tones alone, suggesting an implicitly learned association with the odours (Arzi et al. 2012). Another study showed that conditioned responses can be extinguished during sleep (Hauner et al. 2013) (see also chapter by Shanahan and Gottfried). Human subjects underwent olfactory contextual fear conditioning during wake. Re-exposure to the odorant context during slow-wave sleep promoted stimulus-specific fear extinction, with parallel reductions of hippocampal activity and reorganization of amygdala ensemble patterns. This somewhat unintuitive finding may be understood considering sleep's role in dampening the emotions associated to a memory (Talamini et al. 2013; Gujar et al. 2011; van der Helm et al. 2011b; Yoo et al. 2007; Pace-Schott et al. 2011; Deliens et al. 2012; Wassing et al. 2016) (see also chapter by Cunningham and Payne). Sleep may thus benefit declarative consolidation, while reducing memories' emotional tone (Hofman et al. 2010).

A possible explanation for the differential results of studies investigating new learning during sleep may again be related to the neural networks underlying memory formation for the presented materials. While the successful conditioning and fear extinction attempts probably relied on subcortical and allocortical brain circuitry, the failure to establish memories for more complex stimuli, including verbal material and real world sounds, may be related to their dependence on neocortex.

Neural Mechanisms Underlying Targeted Memory Manipulation During Sleep

The results of targeted memory manipulations during sleep, suggest that at least part of sleep's benefits for memory are due to processes that act at the local level, on specific memories or parts thereof. Given replay findings in rodents (Peyrache et al. 2009; Ji and Wilson 2007; Atherton et al. 2015) and some limited evidence regarding synaptic plasticity during sleep (Sadowski et al. 2016; Frank 2015), a plausible hypothesis holds that the benefits arise through cue-induced reactivation of specific memory representations, leading to synaptic modifications that stabilise the pertaining ensembles. Direct evidence for this putative mechanism has been notoriously difficult to obtain. However, recent experiments, some using advanced experimentation techniques, have now provided convincing support.

In one of these studies, rats were trained on a sound to location association task, while neurons in the hippocampus were being recorded. Presentation of the sounds during subsequent sleep, biased reactivation events toward replaying the spatial representation associated with the pertaining cues. These results showed that the content of neuronal sleep replay can be manipulated, in a stimulus-specific manner, by external stimulation (Bendor and Wilson 2012).

A study in our lab on human subjects addressed this same issue at the macroscopic level (Cox et al. 2014a). During learning of word–location associations, words presented in the *left* and *right* visual hemifields were paired with different odours. Presentation of a single odour during a subsequent nap, aimed to selectively reactivate the subset of the studied material presented in the associated hemifield. We found topographically restricted fast spindle responses to memory cues, over posterior parietal areas contralateral to the cued hemifield, i.e. the areas where the visuospatial location of the reactivated material is know to be represented (Fig. 4). These results showed that memory cues can specifically reactivate associated memories, reflected in amplified fast spindling in the cortical area where the memory is stored. The combined experiments provide strong evidence that external cues can indeed reactivate specific, cue-associated memory representations at the level of both the hippocampus and neocortex.

While the two aforementioned studies link sleep-cueing to memory reactivation, two other studies, in rats, addressed the next step in the hypothesised sleep consolidation mechanism, asking whether sleep-reactivation of specific memories can lead to their modification (Barnes and Wilson 2014; de Lavilleon et al. 2015). In one of these studies, hippocampal CA1 was recorded while rats were exploring a maze. During subsequent sleep, the spontaneous activity of a well-identified place cell was used to trigger stimulations of the medial forebrain bundle (MFB) and, therewith, a neurochemical reward state (de Lavilleon et al. 2015). This closed-loop procedure created a strong post-sleep place preference for the associated place field in the maze. The study thus showed that place cells' reactivation activity during sleep still conveys relevant spatial information, which is functionally significant for navigation. Moreover, the findings suggest that existing place memories can undergo lasting modification during sleep. This latter point should be considered with some caution, as the artificial medial forebrain bundle stimulation, which likely increased acetylcholine and dopamine transmission, may induce a plasticity state that does not naturally occur during sleep.

The other study used olfactomimetic electrical stimulation of olfactory neurons in piriform cortex to create artificial odour memories (Barnes and Wilson 2014; see Fig. 5 of the chapter by Wilson, Kondrakiewicz and Barnes). Two different stimulation patterns were used as CS+ and CS−, respectively, in a fear conditioning procedure. Imposed replay of artificial olfactory memories during post-training SWS enhanced the strength of fear conditioning upon subsequent awake testing, suggesting that the cue reactivated the concomitant memory of the unconditioned fear stimulus and the association was strengthened. On the other hand identical replay during waking, as expected, induced fear extinction. Interestingly, imposed SWS replay of either the CS− or a random olfactomimetic stimulus, induced

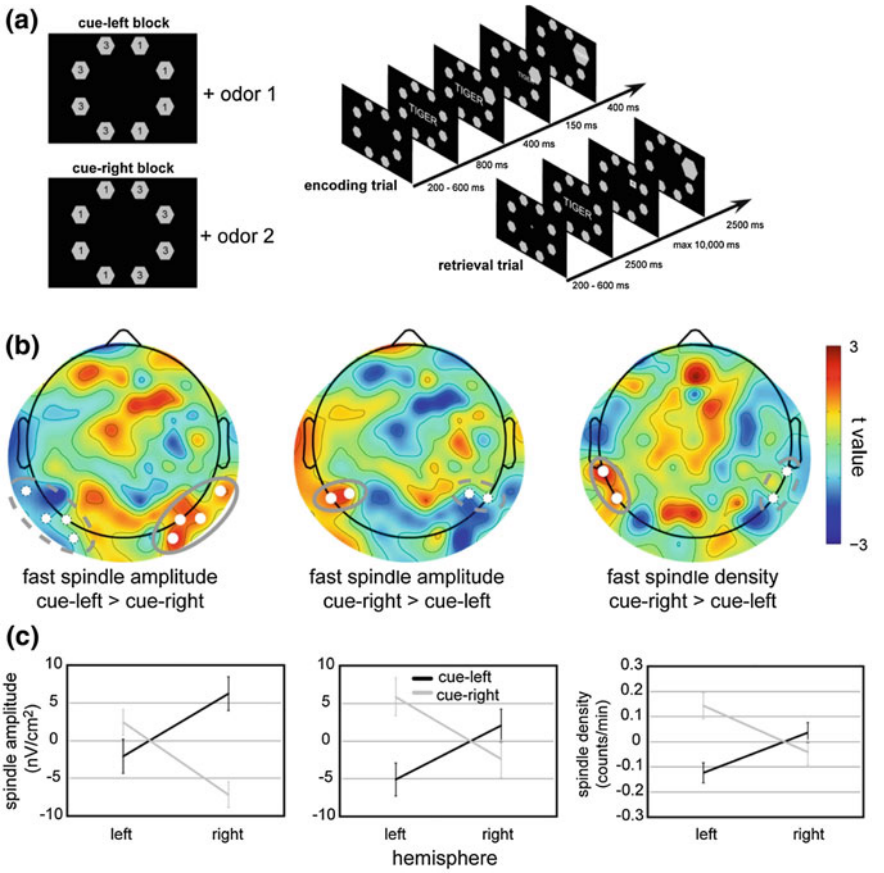


Fig. 4 High-density sleep-EEG with 128 channels was applied to show local sleep spindle modulations in relation to reactivation of specific memories (Cox et al. 2014a). **a** During learning of word-location associations, words presented in the left and right visual hemifields were paired with different odours. During a subsequent nap, a single odour was presented to selectively reactivate a subset of the studied material in sleeping subjects. *Left panel*: Schematic representation of word-location assignments for cue-left (*top*) and cue-right (*bottom*) word blocks. The numbers in each of the 8 locations indicate how many words were associated with that position. A block consisted of 16 words in total and was consistently paired with one odour. *Right panel*: Encoding (*top*) and retrieval (*bottom*) trial timing. **b–c** The reactivation of *left-sided* and *right-sided* stimuli resulted in differential fast spindle modulation topographies, with increased fast spindle amplitude (*left* and *middle panel*) and fast spindle density (*right panel*) in parieto-occipital areas contralateral to cueing side. **b** T maps showing significant cueing side effects. *Grey solid ovals mark* significant clusters; significant electrodes in each cluster are shown as *white dots*. *Grey dashed ovals* depict contralateral 'mirror' clusters used for assessing hemisphere-dependence of cueing side effects in (**c**). **c** Significant crossover interactions between cueing side and hemisphere for all clusters indicate bilaterally symmetrical spindle modulations in response to memory cues

generalisation of the fear memory to those artificial patterns. This is in line with the notion that information can be recombined during sleep replay (Gupta et al. 2010), possibly promoting new insights, problem solving and other creative processes. Of note, the main finding of this experiment appears opposite to that of the olfactory contextual fear conditioning experiment in humans, described in the previous section (Hauner et al. 2013). While the reason for this discordance is not apparent, it might be noted that the main effect in the rat study is considerably larger than that in the human study.

Targeting Memory Manipulations to Receptive Sleep Windows

As indicated earlier, some of the strongest evidence regarding episodic memory consolidation points to SWS and, at a more fine grained level, to temporally coupled slow oscillations (Marshall et al. 2006; Marshall et al. 2011), sleep spindles (Gais et al. 2002; Clemens et al. 2005; Clemens et al. 2006; Schmidt et al. 2006; van der Helm et al. 2011a; Cox et al. 2012; Kaestner et al. 2013; Mednick et al. 2013), and sharp-wave ripples. At first glance, signs of overall synchronised neural activity and reduced functional connectivity in cortical networks during SWS, seem in discordance with the idea that coordinated, large scale neural processes could be taking place. However, studies with higher temporal resolution suggest that neural networks during deep sleep fluctuate between two states, regulated by the phase of slow oscillations. Indeed, membrane potentials of neocortical circuits alternate between depolarized up-states and hyperpolarized down-states. The slow oscillation up-states hold most of the faster activity (Mölle et al. 2002; Cox et al. 2014b; Valderrama et al. 2012), are related to higher excitability (Bergmann et al. 2012) and plasticity (Hoffman et al. 2007), and feature complex interregional functional coupling of cortical networks (Cox et al. 2014b) (Fig. 1). On the other hand, all these characteristics are strongly reduced during the hyperpolarised down-states. In view of the above, slow wave up-states may represent windows of opportunity for memory consolidation and perhaps other forms of higher order information processing, while down-states reflect a deeply inhibited network state. As such, memory manipulations during sleep may work best if they are targeted to slow wave up-states.

To investigate this intriguing possibility, we recently developed a closed-loop procedure to target stimuli to any selected phase of on-going slow oscillations (Fig. 5) (Cox et al. 2014c). This procedure can be used for precise manipulation of the sleeping brain, while simultaneously brain activity is recorded at high spatial and temporal resolution through high-density EEG polysomnography. Phase targeting allows stimuli to be presented repeatedly, while being consistently time-locked to a specific oscillatory phase. This enables new and sophisticated experimentation. For instance, if indeed slow oscillations regulate windows of opportunity for neocortical information processing, a memory cue for neocortically-based information may need

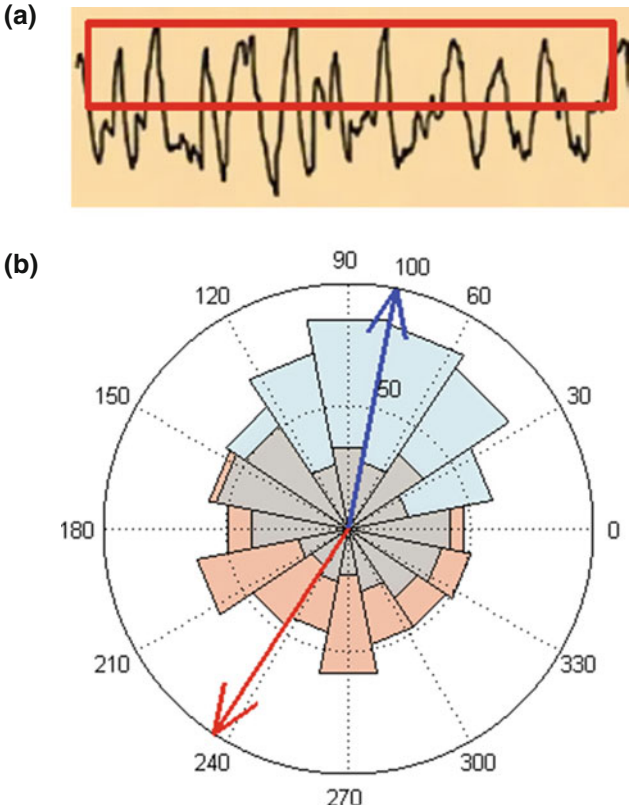


Fig. 5 Cox and colleagues developed a technique for presenting stimuli phase-locked to ongoing EEG oscillations (Cox et al. 2014c). **a** The procedure was used to present real world auditory stimuli phase-locked to either slow oscillation up-states (*red box*) or down-states, to test differential information processing in these two states (**b**) Phase targeting performance for up-state (*blue*) and down-state (*red*) of slow oscillations is shown in a rose plot (averaged over 12 subjects; N = 171). *Arrows* indicate average phase angle; 90° corresponds to the peak of the up state, 270° to the trough of the down state. Significant clustering of auditory stimulus presentations to the up-state (*blue*) or down-state (*red*) of slow oscillations was demonstrated (Rayleigh Test for 'non-uniformity' $*P < 1e{-}13$)

to arrive sufficiently often in the right slow oscillation phase in order to boost consolidation of the cued memory. After all, the build-up of lasting memory representations is a function of the number of times a representation has been activated. Similarly, repeated phase-optimized stimulus presentation could benefit memory modification or the induction of new learning. An ulterior benefit of phase-locked stimulation is that phase effects on neural processing can be analysed much more effectively than with random stimulation and post hoc stimulus sorting, as trials will be more tightly grouped in the phase(s) of interest and arbitrary stimulus sorting criteria are avoided. Furthermore, targeting of stimuli to optimized receptive

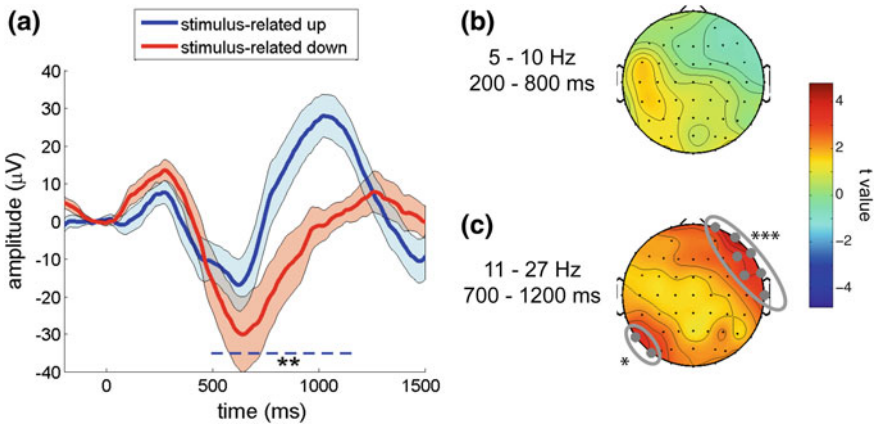


Fig. 6 Slow oscillation phase-dependent stimulus processing. **a** Differential stimulus-evoked waveforms for up (*blue*) and down (*red*) state-presented sound stimuli for frontal channel Fz. **b** Early stimulus-evoked theta power (coinciding with induced down-states) did not differ reliably between up- and down-targeted stimuli. **c** Late spindle/beta power (coinciding with induced up-states) was higher for up-targeted sounds than for down-targeted stimuli across the entire scalp, reaching significance in a right fronto-temporal area (electrodes Fp2, F8, FC6, T8, AF8, F6 and FT8), and a left parietal region (P7 and PO7). Reliable differences are indicated with: * $P < 0.025$; ** $P < 0.01$; *** $P < 0.001$. (From Cox et al. 2014c)

windows avoids unnecessary sleep disturbance with stimuli that have a poor chance of leading to memory change. Thus, stimulus locking to slow oscillation phase maximizes the opportunity to demonstrate slow oscillation phase-dependent learning and memory processing.

In a first study using this technique, we examined how cortical networks respond to real-world sound stimuli as a function of slow oscillation phase (Cox et al. 2014c). The sounds were repeatedly presented to sleeping subjects, targeted to either slow oscillation up or down-states, with a consistent sound to phase relation. Brain-wide responses to up and down-state stimuli, corrected for spontaneous slow oscillation dynamics, were evaluated. Up-state sounds induced a second up-state that occurred sooner in time, had higher amplitude, and featured enhanced spindle and beta activity (Fig. 6). Responses were relatively widespread, but largest over frontal cortex, the area that also provided the signal for phase targeting (Fz). These findings suggest enhanced stimulus processing with up-state-targeted stimulation.

Of note, both up- and down-state stimuli evoked K-complex-like responses, i.e. high-amplitude EEG patterns that are in many ways similar to slow oscillations. Compared to a no-stimulation condition, stimulation enhanced theta power around 300 ms after stimulation onset, corresponding to the sharp K-complex 'down-state', and induced a spindle/beta boost during the subsequent up-state, from 500 ms onwards (Fig. 7). In line with these findings, two other studies also found that the presentation of stimuli during sleep, be they simple tones or meaningful stimuli, was accompanied by a temporary rise of power in the theta (Schreiner and Rasch 2014)

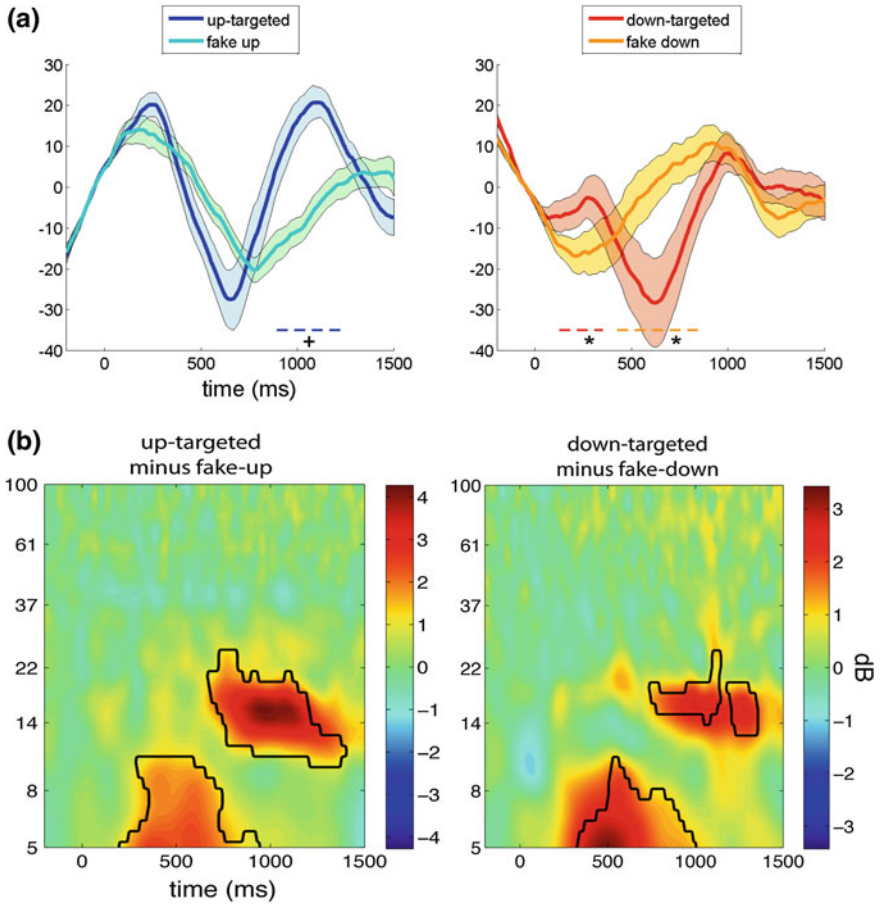


Fig. 7 Grand-average event-related potentials (**a**) and time-frequency power plots (**b**) for slow oscillation up-state and down-state-targeted auditory stimuli during sleep for frontal channel Fz. **a** Up-targeted (*left panel*) and down-targeted (*right panel*) events are shown against 'fake up' and 'fake-down' events, respectfully. The fake events are non-stimulated slow oscillations, where the target phases are matched to those of the stimulation events. *Error shading* indicates standard error of the mean. *Dashed coloured lines* near *bottom* signify time period of significant difference at cluster-level, *with colour* indicating the more positive waveform (+$P < 0.05$; * $P < 0.025$; ** $P < 0.01$; *** $P < 0.001$). **b** Time-frequency power difference plots corresponding to the *upper ERP panels*. Both up-targeted stimulus delivery compared with fake-up events (*left*) and down-targeted sounds compared to fake down events (*right*) elicit theta and spindle/beta activity relative to fake-down events. The spindle response is larger for the up-state stimulation condition. (From Cox et al. 2014c)

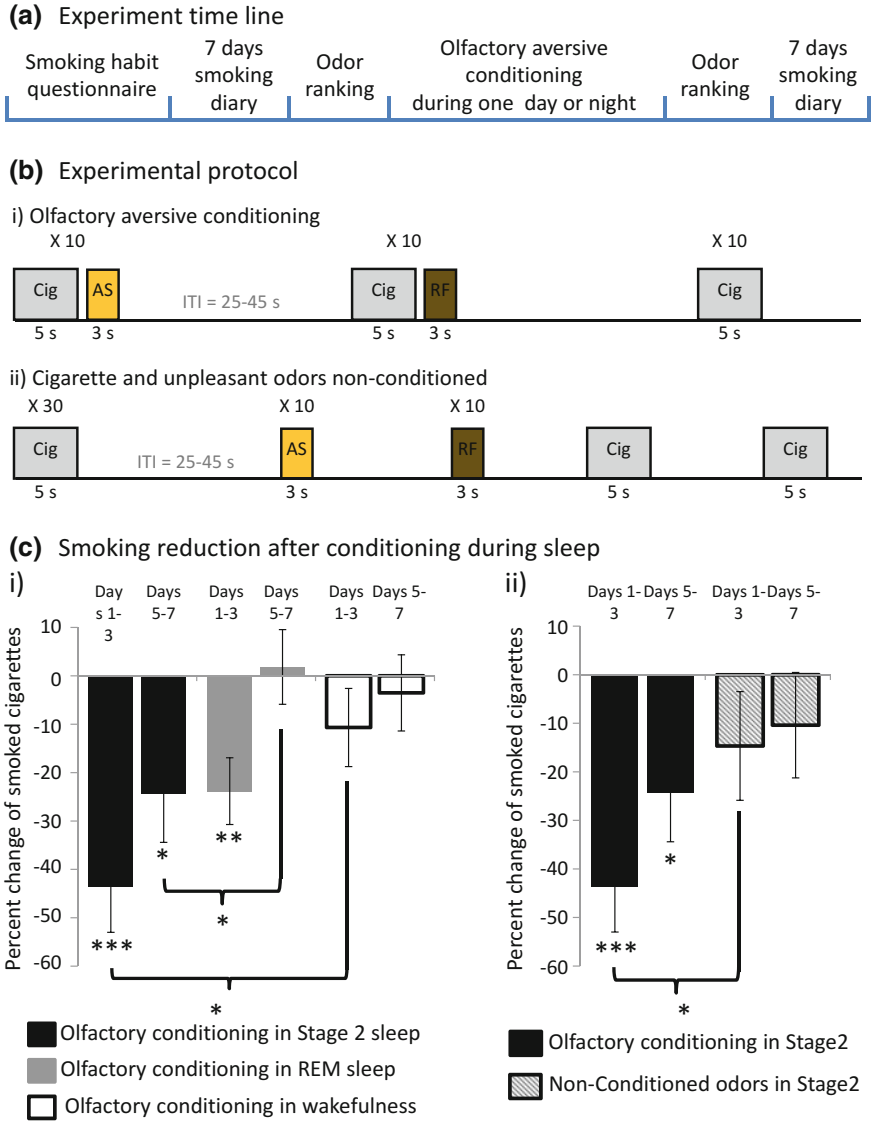
and spindle/gamma range (Ngo et al. 2013b; Schreiner and Rasch 2014). The induced theta power (in either phase condition) may directly reflect the induction of the K-complex like down-state, as the frequency of this relatively sharp deflection encompasses the low theta range.

Fig. 8 **a** Experiment time line. **b** (i) The main experimental protocol. Olfactory aversive partial-reinforcement trace conditioning between cigarette odour (Cig) and unpleasant odours. Stimuli were generated in blocks of 30 trials: 10 reinforced trials with unpleasant odour of ammonium sulphide (AS) (*yellow*), 10 reinforced trials with unpleasant odour of rotten fish (RF) (*brown*) and 10 non-reinforced trials (cigarette odour alone) (*grey*). (ii) The non-conditioned control protocol. Cigarette and unpleasant odour administration in randomized order such that the cigarette odour and unpleasant odours were non-conditioned. **c** (i) Percent change in smoked cigarettes in the first (days 1–3) and second half (days 5–7) of the experiment following conditioning during stage 2 sleep (*black*), REM sleep (*grey*), and wake (*outline*). (ii) Percent change in smoked cigarettes in the first (days 1–3) and second half (days 5–7) of the experiment following olfactory aversive conditioning (*black*), and non-conditioned odours (*striped*) administration during Stage 2 sleep. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$. (From Arzi et al. 2014) ▶

Although up-state stimuli in our study induced a network state that should be conducive to information processing and plasticity, enduring memory for the presented stimuli was not observed. Thus, it may not be possible to induce entirely new declarative memories during sleep. Alternatively, the limited number of presentations per stimulus (±3) in this nap study may not have been sufficient to effectuate notable memory build up. Current closed-loop experiments in our lab employ more frequent stimulation and use the procedure to address various aspects of sleep-related memory processes, including the possibility to enhance or depress sleep-related memory consolidation through phase-locked presentation of reactivation cues.

Closed-loop procedures, such as the one described above, present new and powerful experimental tools that are generating considerable interest in the field of sleep research. In general, closed-loop procedures allow the presentation of stimuli in alignment with specific patterns in on-going biophysical signals. The methods typically involve fast algorithms performing near real-time analysis of the pertaining signal, to detect the pattern of interest, and fast hardware/software loops to deliver stimuli in temporal concordance with a detected pattern.

In sleep and cognition studies, the first closed-loop methods were used to interrupt sharp wave-ripples in rodents (Girardeau et al. 2009; Ego-Stengel and Wilson 2010). Next, procedures in humans were developed to present auditory stimuli to particular slow oscillation phases (Ngo et al. 2013a, b; Cox et al. 2014c; Bergmann et al. 2012; Santostasi et al. 2016). The first of these phase-targeting methods was based on a simple, inflexible, procedure that does not adapt to ongoing fluctuations in slow oscillation frequency (Ngo et al. 2013a, b; Bergmann et al. 2012) and has not been validated in terms of phase targeting accuracy. Our own (Cox et al. 2014c) and another, very recently reported, method (Santostasi et al. 2016) are based on modelling and predicting on-going slow oscillation activity. These methods do adapt to the natural fluctuations in slow oscillation frequency and have, at least to some extent, been validated. Ongoing developments in our lab have recently resulted in a new, thoroughly validated, closed-loop stimulation procedure for oscillatory phase targeting, which is faster and more accurate than any previously reported method (Talamini et al. 2016). The new procedure enables interference with oscillatory dynamics much faster than the slow oscillation, opening up new and exciting research possibilities both within and beyond the field of sleep.



Applications

As discussed in previous sections, sleep-related memory manipulations have brought forth crucial evidence regarding the role of sleep in cognition. However, they also instigated the exiting idea that sleep's hidden processing potential might be harnessed for clinical and educational purposes. It might, for instance, be possible to modify traumatic memories, extinguish unadaptive behaviours, or even instil entirely new thoughts and ideas.

Concrete steps toward clinical application regard the use of aversive conditioning during sleep to reduce addictive behaviour in cigarette smokers (Fig. 8) (Arzi et al. 2014). Specifically, cigarette smoke odour was paired with a highly aversive odour in a partial conditioning protocol during either wake, stage 2 sleep or REM sleep. Conditioning during sleep, in particular during stage two, reduced cigarette smoking in the week after the conditioning night by approximately 30%, on average. Conditioning during REM was less effective (11% reduction) and conditioning during wake was not effective at all. The effects of conditioning tapered off in the course of the post-conditioning week. Nevertheless, these initial observations following just one conditioning night mark sleep-related conditioning as a promising strategy in the treatment of addiction.

Other possible clinical applications of sleep-related memory manipulation regard the modification of trauma memories. Perhaps the presentation, during sleep, of cues associated to beneficial therapy sessions, or 'safe circumstances', could strengthen these memories and benefit recovery. Alternatively, it might be possible to selectively 'erase' maladaptive memories, or to restore, sleep's normal role in the reduction of memories' emotional tone. Reports on sleep-related memory manipulation thus far mainly concern neutral memory. However, studies evaluating emotional memory manipulation during sleep are underway and will be informative as to possible applications in the emotional realm.

A different type of clinical application regards the possible treatment of sleep problems with external stimulation. Sleep problems have very high prevalence in modern societies, and many sleep disorders feature reduced SWS. While pharmacological treatments are available, these do not work in all people or in all conditions. To give just one example, no treatments with proven efficacy currently exist for PTSD-related sleep disorder. Moreover, sleep-promoting drugs, even when they do improve sleep, often do not restore normal sleep physiology. The benzodiazepines, for instance, have REM supressing effects. Finally, as with all pharmaceutical drugs, sleep medication has side effects, including paradoxical effects on sleep and risk of addiction. A new, non-pharmacological form of treatment would therefore be of considerable societal interest.

The applicability of external stimulation to this kind of goal remains to be seen. A recent study indicates that the responsiveness of healthy sleep to up-state targeted stimulation is self-limiting (Ngo et al. 2015). That is, boosting, in terms of slow oscillation amplitude and high frequency power content, only works for one or two oscillations in a row and then quickly fades out. In line with this finding, previously reported effects of external stimulations on sleep were all observed closely following stimulation; no studies have reported alterations in the amount of SWS or other sleep macrostructural parameters. It should, however, be considered that these findings were achieved with random stimulation or using an unvalidated slow oscillation phase targeting method with uncertain accuracy (Ngo et al. 2015). It will be interesting to see whether methods with demonstrated phase-targeting precision will lead to more favourable outcomes. Also, stimuli besides auditory ones, for instance electromagnetic fields, could be considered for this type of development.

Moving from clinical to educational possibilities, an application that seems close at hand concerns the use of sleep manipulations to aid learning. In particular, the existing literature on sleep-related memory manipulations suggests it should be possible to enhance the retention of information studied prior to sleep. A consideration here is that the memory effects in the studies thus far were typically small and memory benefits were not observed in all studies. It thus remains to be seen whether procedures can be developed in which the size and robustness of effects will be sufficient to be of practical interest. Also in this case, the targeting of stimuli to potentially receptive sleep windows, such as the slow oscillation up-state, may increase the effectiveness of sleep-related memory manipulations. As such, possible applications towards the enhancement of learning certainly merit investigation.

Concluding Remarks

Approaches in which the candidate process components of memory consolidation have been actively manipulated, have led to great advances in the field of sleep and memory. On this journey, sophisticated techniques have been developed to overcome the challenges posed by investigating brain activity in a state that is devoid of behavioural output. Clearly, the field is as yet at an early stage and many unknowns regarding sleep-related memory processing remain. This is especially the case considering the broader role of sleep in cognition, which, besides memory consolidation, involves more flexible information processing (Gupta et al. 2010) leading to insights (Wagner et al. 2004; Cai et al. 2009; Beijamini et al. 2014), as well as a central role in emotional memory regulation (Talamini et al. 2013; Gujar et al. 2011; van der Helm et al. 2011b; Yoo et al. 2007; Pace-Schott et al. 2011; Deliens et al. 2012; Wassing et al. 2016). Similarly, the multiple factors determining the success of memory manipulations during sleep have yet to be clarified. However, the tools, basic knowledge and broad interest from the research community are in place to support fast progress in this field, both at the level of fundamental advance and in terms of applications.

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