The sleeping brain switches between working memory and long-term memory processing.

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Abstract

Both working memory (WM) and long-term memory (LTM) utilize non-rapid eye movement (NREM) sleep for improvement. LTM systems consolidation is supported by hippocampal-cortical communication, whereas WM improvement is associated with the strengthening of prefrontal-autonomic networks. Prior studies have demonstrated that these two networks demonstrate mutual antagonism during sleep; but this trade-off has not been confirmed in human sleep and its functional significance is unknown. Here, we investigated the functional impact of central and autonomic activity on LTM and WM improvement. We pharmacologically enhanced central activity and observed targeted suppression of autonomic activity, and using effective connectivity, we showed greater causal influence of central over autonomic activity. Finally, we demonstrated that the central and autonomic antagonism was reflected in a behavioral trade-off between overnight LTM and WM processing. These results suggest that NREM sleep confers benefits to working and long-term memory by switching between autonomic and central processing.

Key words: Working Memory, Long-Term Memory, Vagal Activity, Sleep spindles, Sigma Activity, Sleep

Main text

Introduction

Working memory (WM) and long-term memory (LTM) serve separate functions. The former is a control process for planning and carrying out behavior that is information-independent, whereas the latter is an information-dependent vast store of knowledge and record of prior events. Although some have argued that these memory domains are separate systems that are supported by distinct neural substrates, the prefrontal cortex (PFC) (Funahashi and Kubota, 1994) and hippocampus (Squire & Zola-Morgan, 1991), respectively, recent results have shown overlap in brain regions activated during tasks probing LTM and WM (Baddeley & Hitch, 1974) (Ranganath et al., 2003). Moreover, performance in one task influences performance in the other (Lugtmeijer et al., 2019; Sandry et al., 2020), and subtle but noticeable long-term memory deficits have been reported in patients with prefrontal brain lesions (D'Esposito & Grafman, 2019). These findings are consistent with the suggestion that working and long-term memory may be part of a single system that can functionally differentiate information storage for different goals (Ranganath & Blumenfeld, 2005). They also underscore the need to determine shared and distinct fundamental mechanisms underlying these two types of memory.

Both WM and LTM rely on sleep to facilitate performance improvement. According to the framework of systems consolidation, long-term memories are initially processed by a fast learning system in the hippocampus that binds information into transient representations (i.e. encoding). With repeated reactivation, the memories eventually become less reliant on the hippocampus and more stabilized in long-term cortical stores (i.e., consolidation). Converging evidence suggests the sleep may be an optimal offline period for consolidation as it facilitates dialogue between the hippocampus and the neocortex (Mednick et al., 2011; Rasch & Born, 2013). Specifically, during non-rapid-eye-movement (NREM) sleep, the memory trace is replayed during hippocampal sharp wave ripples (SPW-R) that are nested within thalamic sigma oscillations, suggesting replay as a mechanism for information transfer between neocortical and hippocampal cell assemblies. Sigma activity is thought to reflect this replay mechanisms as experimental interventions that boost sigma in humans enhance hippocampal long-term memory (Lustenberger et al., 2016; Mednick et al., 2013; Wamsley et al., 2013; Zhang et al., 2020), suggesting a causal role for sigma activity in LTM consolidation.

Classic models of WM propose a mechanism for online information maintained by elevated firing of prefrontal neurons (Funahashi et al., 1989) and an supervisory executive control process supported by a prefrontal-subcortical inhibitory network (Compte et al., 2000; Funahashi et al., 1989; Fuster & Alexander, 1971). This circuitry innervates the heart via sympathetic stellate ganglia and parasympathetic vagal nerve efferents. As such, cardiac autonomic activity is thought to reflect effective functioning of prefrontal control processing (Thayer et al., 2009). Accordingly, vagally-mediated, high frequency heart rate variability (HF HRV, 0.15-0.4 Hz) during wake correlates with executive function tasks that rely on PFC activity (Thayer et al., 2009), including WM (operation-span task: Mosley et al., 2018; n-back task: Hansen et al., 2003). Recent studies have shown that WM improvement only occurs when the interval between training sessions contains nocturnal (Zinke et al., 2018; Kuriyama et al., 2008) or daytime sleep (Lau et al., 2015). Given that NREM sleep is a period of vagal dominance compared with wake, a recent study identified vagal HF HRV during NREM as a strong predictor of WM improvement (Chen, Whitehurst, et al., 2020a).

The overall picture emerging is that NREM sleep supports improvement in WM via strengthening of prefrontal-autonomic inhibitory networks, as well as the formation of LTM via thalamocortical sigma activity. The question is how the sleeping brain performs both of these complex feats? Prior research suggests a potential antagonistic relation between the two neural processes during NREM sleep. Using ripple-triggered fMRI in monkeys, Logothetis and colleagues showed that ripples orchestrate a privileged state of enhanced central brain activity that may serve to boost communication between hippocampus and cortex by silencing output from the diencephalon, midbrain and brainstem (Logothetis et al., 2012), regions associated with autonomic regulation. On the other hand, stimulating the locus coeruleus (LC), an area thought to mediate propagation of vagal nerve activity to higher-order neural regions, blocks the generation of ripple-associated sigma activity and causes hippocampal-dependent spatial memory deficits (Novitskaya et al., 2016). These studies suggest a potentially antagonistic interplay between central sigma-dependent and autonomic vagal-dependent processing. However, the functional significance of this trade-off has not been studied.

In the present study, we enacted a pharmacological strategy to investigate the bi-directional interplay between central sigma-dependent and autonomic vagal-dependent processing during overnight sleep and its impact on LTM and WM. Considering a potential antagonistic link between sigma and vagal activity, we pharmacologically increased sigma

activity during overnight sleep and measured changes in vagal activity, sigma-vagal interaction, and long-term vs working memory performances. We report data from two double-blind, placebo-controlled, cross-over experiments. In experiment 1, we pharmacologically boosted sigma activity using zolpidem (an GABA-A agonist hypnotic) and tested the impact on vagal autonomic activity across sleep stages. In particular, we computationally tested our model that central sigma activity would suppress autonomic vagal activity using effective connectivity (the influence that one node exerts over another under a network model of causal dynamics; Friston, 1994, 2011). We hypothesized that 1) zolpidem would enhance sigma activity during NREM sleep, 2) zolpidem would decrease vagal activity during sleep, and 3) sigma increases would boost information flow exerted from central over autonomic regions. In experiment 2, we aimed to assess the functional importance of sigma-guided vagal suppression in the trade-off between long-term and working memory. We hypothesized that 1) central-autonomic patterns during sleep from Experiment 1 would be replicated, 2) sigma-guided vagal suppression would result in parallel behavioral effects with greater long-term memory and worse working memory, and 3) the degree to which changes in the magnitude and the direction of information flow between central and autonomic systems would affect the behavioral trade-off between long-term and working memory.

By exploiting a hypnotic's amplification of sigma during NREM sleep, we identified a novel antagonistic relationship between central sigma and autonomic vagal activity during sleep, which predicted a trade-off between long-term memory consolidation and working memory efficiency. These results suggest that NREM sleep confers benefits to working and long-term memory by switching between vagally-mediated and sigma-meditated processes. Furthermore, this sleep switch can be biased towards long-term memory consolidation by increasing sigma activity, in this case pharmacologically, and presumably by other methods as well. We determined that effective connectivity between central and autonomic nervous systems was dominated by central activity, and could be even further biased when sigma was pharmacologically increased, with ensuing functional changes to LTM and WM. These results illuminate mechanisms for working and long-term memory processes during sleep that may have implications for understanding fundamental questions regarding their shared processes.

Results

Experiment 1.

Based on previous findings (Logothetis et al. 2012), we predicted that central sigma power would have an inhibitory effect on cardiac vagal tone. To this end, we administered zolpidem in a double-blind, placebo-controlled, randomized cross-over design, in which each participant experienced two nights per drug condition (zolpidem or placebo; a total of 4 nights; M_{age} = 20.88 ± 1.88 years, 17 Females), with EEG and ECG monitored (Figure 1 shaded area). The order of drug conditions was counterbalanced with at least a one-week interval between the experimental visits to allow for drug clearance. We performed power spectral analysis to quantify normalized sigma activity and analyzed HRV profiles. Our intervention was successful, whereby zolpidem enhanced sigma activity during stage 2 sleep (central channels: t = 2.112, p = .0349; parietal channels: t = 2.214, p = .0270, corrected by Tukey's multiple comparisons), consistent with prior literature (Mednick et al., 2013; Zhang et al., 2020).

As we hypothesized, zolpidem not only increased sigma activity, but also selectively suppressed vagal tone during sleep, measured by RMSSDln (Figure 2a) and high-frequency

HRV (HFln; Figure 2b), but had no impact on low-frequency HRV (LFln; Figure 2c). Statistics and Graphs for RRmean, Total Power (TPln), and HFnu are presented in Supplemental Figure S1. Means and standard errors for the HRV variables across sleep stages are provided in Supplemental Table S4.

We then tested our hypothesis that central sigma power would have greater causal influence on vagal autonomic activity than the opposite direction, and such difference would be increased by zolpidem. To test this prediction, we used effective connectivity estimation (Figure 3a). In particular, we tested the hypotheses that central sigma naturally exerts greater causal influence on autonomic vagal activity in the placebo condition, and that increasing sigma with zolpidem would increase causal information flow from sigma to vagal activity, while decreasing the causal information flow from vagal to sigma activity in the zolpidem condition. For each subject, we calculated two measures: HFOutflow and HFInflow (see Methods). We confirmed our hypothesis that central sigma power exerted greater flow on vagal activity than the opposite direction in the placebo condition ($p < .0001$; Figure 3b). We also confirmed that such difference would be increased by zolpidem, with an increasing causal effect from sigma to vagal activity ($p = .0369$; Figure 3b). Next, we calculated a composite score, the effective connectivity ratio: HFInflow over HFOutflow, where higher numbers represented greater central sigma control over autonomic vagal activity. We observed a higher effective connectivity ratio during the zolpidem night ($p = .0059$). Taken together, Experiment 1 verified our hypotheses that central activity naturally exerts dominance over autonomic activity during NREM sleep, and that increasing sigma activity via zolpidem inhibits vagal activity to a greater extent and enhances central sigma control over autonomic vagal activity.

Experiment 2.

In an independent sample of participants ($N = 38$; $M_{age} = 20.85 \pm 2.97$ years;19 Females), we added a behavioral experiment (Experiment 2; Figure 1) to the original design of Experiment 1 (Figure 1 shaded area) to test whether we could replicate the physiological results of Experiment 1 and determine their functional importance for sleep-dependent cognition. Again, we exploited zolpidem to modulate the interaction between central sigma and autonomic vagal activity, and examined its impacts on the improvements of long-term memory and working memory (Figure 1). The order of drug conditions was counterbalanced with at least a one-week interval between the two experimental visits to allow for drug clearance. Due to the pharmacodynamics of zolpidem, which has a half-life of (1.5–4.5 h), and onset (mean *T*max 1.6 h; Drover, 2004), we divided the night into quartiles and focused our analyses on quartile two and three combined in order to maximize differences in drug conditions. We hypothesized that sigma-guided vagal suppression effects would result in parallel behavioral effects with greater long-term memory and reduce improvement in working memory. We further hypothesized that the magnitude and the direction of causal information flow between central and autonomic systems would be correlated with the trade-off between long-term and working memory.

The physiological results across one night of sleep in Experiment 2 were consistent with those from Experiment 1. We confirmed that zolpidem increased sigma activity during sleep while suppressing vagal tone, measured by RMSSDln and high-frequency HRV (HFln), but had no impact on low-frequency HRV (LFln). Statistics and Graphs for HRV variables were shown in Supplemental Materials (Supplemental Figure S2 and Table S5). Similarly, we replicated the effective connectivity results (Figure 3c), in which zolpidem increased effective connectivity

ratio (p = .0265), indicating greater causal influence of central sigma activity on autonomic vagal activity.

We further assessed the functional roles of each physiological measures (EEG sigma activity, cardiac vagal activity, and effective connectivity ratio) on long-term and working memory changes across sleep. We hypothesized that increasing central sigma activity would benefit LTM retention in a word-pair-associates task, whereas decreasing autonomic vagal activity would hinder WM improvement on a working memory operation span task. To this end, we examined overnight and 24-hr change scores in each task between the two drug conditions. For the word-pair task, our analysis showed that zolpidem significantly increased 24-hr LTM retention (Figure 4a right panel) and overnight retention (Figure 4a left panel). For the working memory operation span task, our analysis demonstrated that zolpidem decreased overnight improvement (Figure 4b left panel) and 24-hr improvement (Figure 4b right panel), compared to placebo. In summary, we confirmed our behavioral hypothesis that sigma-guided vagal suppression would increase long-term memory (Figure 4a) and decrease working memory improvement (Figure 4b). See Supplemental Figure S1 and Table S8 for behavioral summary statistics.

Next, we tested the correlations between each physiological measure (EEG sigma activity, cardiac vagal activity, and effective connectivity ratio) and memory changes across sleep using Pearson's correlation coefficients. We found a functional dissociation in vagal activity and behavior, where vagal activity during SWS was negatively correlated with LTM in the zolpidem condition (24-hr retention and HFln: r = -.460; p = .018; Figure 4c right panel), and positively correlated with WM improvement (overnight retention and HFln: r = .422; p = .032; Figure 4c left panel) in the placebo condition. Correlational statistics between vagally-mediated HRV parameters and behavioral improvements are shown in Supplemental Materials (Supplemental Table S9). No significant correlations were found between EEG sigma activity and WM improvement (zolpidem: all ps > .5687; placebo: all ps > .1943) or between EEG sigma activity and LTM retention (zolpidem: all ps > .15516; placebo: all ps > .1383). Taken together, vagal activity was positively associated with WM improvement, but inversely related to LTM.

We, then, asked whether central and autonomic antagonism impacted the trade-off between LTM and WM improvement by correlating the effective connectivity ratio with the normalized LTM-WM difference score, where higher numbers represent greater LTM than WM improvement. We found a positive correlation between the effective connectivity ratio and normalized LTM-WM difference score in the zolpidem (r = .429; p = .020; Figure 4d right panel) and non-significant positive correlation in the placebo condition (r = .251; p = .190; Figure 4d left panel). These results suggested that the more central activity exerted influence on autonomic vagal activity, the more sleep was biased towards sigma-dependent LTM consolidation (and away from vagal-dependent WM processing).

Discussion

The current work identified two distinct mechanisms during NREM sleep that support the enhancement of central sigma-dependent long-term memory (LTM) consolidation and autonomic vagal-dependent working memory (WM) processing. In study 1, we exploited the hypnotic zolpidem to enhance sigma activity during NREM sleep (Mednick et al., 2013) and showed a novel effect of vagal suppression during NREM. Next, we used the effective connectivity estimation technique to test the hypothesis that central sigma activity actively suppressed vagal autonomic activity, and not the other way around. Consistent with our

hypothesis, results showed that central sigma exerted greater causal control over autonomic vagal activity, and that pharmacologically increasing sigma activity boosted causal information flow from central to autonomic channels and decreased flow from autonomic to central channels. In a separate set of subjects, we repeated the pharmacological intervention and tested the functional significance for cognitive performance of this central-autonomic antagonism during NREM sleep by testing LTM and WM before and after a night of sleep. The physiological results from experiment 1 were replicated. Moreover, the sigma-guided vagal suppression was associated with enhanced LTM retention at the cost of reduced WM improvement, with the magnitude of vagal suppression predicting the trade-off between LTM and WM benefits. These findings reveal evidence of a sleep switch that toggles between NREM mechanisms that support central-dependent LTM and autonomic-dependent WM processing. Further, this system can be biased towards greater consolidation by boosting sigma activity.

Sigma activity is proposed to facilitate plasticity by producing long-term changes in responsiveness in cortical neurons (Timofeev et al., 2002) and increasing dendritic Ca^{2+} influxes (Seibt et al., 2017), particularly enhanced when coupled to down-to-up transitions of the sleep slow oscillation (Niethard et al., 2018). Recently, Dickey and colleagues demonstrated the first evidence in humans that sigma activity may promote spike-timing-dependent plasticity (STDP), where correlated pre- and post-synaptic spiking within 25ms windows modulated synaptic strength. STDP within this short window facilitates long-term potentiation (LTP), the cellular mechanism thought to underlie learning and memory (Dickey et al., 2021). Sigma activity, therefore, is thought to promote LTM via cortical synaptic plasticity. Furthermore, at the systems level, hippocampal-dependent long-term memory consolidation has been shown to be supported by replay of memory traces via triple phase coupling of hippocampal sharp-wave ripples nested within thalamic spindles and cortical SOs (Latchoumane et al., 2017). Several different interventions have demonstrated the causal role of sigma and spindle activity on hippocampal-dependent memory consolidation, including pharmacology (Mednick et al., 2013; Niknazar et al., 2015; Zhang et al., 2020), targeted memory reactivation (Antony et al., 2018; Cairney et al., 2018), and transcranial electrical stimulation (Lustenberger et al., 2016), with no benefits to non-hippocampal, procedural learning (Barham et al., 2016). The current findings build upon this prior knowledge by demonstrating that in addition to enhancing hippocampal-dependent memories, sigma also suppressed subcortical vagal activity with significant functional outcomes, specifically a reduction in working memory.

Vagal influence on cognitive function is a core principle of the “Neurovisceral Integration Model” (Thayer et al., 2009), which posits that ANS activity is a peripheral index of the integrity of prefrontal-autonomic networks that support inhibitory, goal-directed, high-order brain functions. The tenth cranial vagus nerve communicates peripheral information to and from the brainstem, with afferents projecting to higher-order, cognitive areas such as prefrontal cortex, anterior cingulate, and amygdala. Additionally, descending projections from the PFC to the brainstem and hypothalamic structures allow for bi-directional communication between the central nervous system and the ANS through the vagus nerve (Packard et al., 1995; Thayer et al., 2009). As such, high levels of vagally-mediated HRV is associated with superior executive function (Williams et al., 2019), working memory (Hansen et al., 2003; Mosley et al., 2018), and emotional regulation (Mather & Thayer, 2018). In older adults with mild cognitive impairment undergoing a six-week training intervention, Lin et al. (2017) revealed a link between autonomic vagal activity and cognitive improvements measured across a range of executive functions including working memory (Lin et al., 2017). They further showed that cognitive training

enhanced HF-HRV and decreased connectivity between striatal and prefrontal regions, suggesting that vagal activity might reflect enhanced cognitive control via greater automaticity and reduced activation between the striatum and prefrontal networks (Lewis et al., 2004). Although sleep was not measured across the cognitive training intervention, the current findings suggest that the strengthening of prefrontal-autonomic networks supporting performance improvement may occur during sleep.

Prominent models of ANS's role in cognition have focused on autonomic activity during waking states, rather than during sleep, which is surprising given that parasympathetic vagal activity is highest during SWS (Bušek et al., 2005; Cellini et al., 2016; Chen, Sattari, et al., 2020; Whitehurst et al., 2018; 2020). Vagal activity is strongly coupled with low frequency, delta activity during SWS and vagal enhancement precedes the onset of SWS (Niizeki & Saitoh, 2018; Rothenberger et al., 2015). Several studies have linked SOs and SWA with WM improvement. For example, studies have shown that fronto-parietal SWA predicts WM improvement (Ferrarelli et al., 2019; Pugin et al., 2015), and Sattari et al. (2019) showed that frontal SOs, but not sigma, predicted WM improvement in older adults (Sattari et al., 2019). However, not all studies report a consistent association between SWA and WM (Chen, Whitehurst, et al., 2020b; Lau et al., 2015; MacDonald et al., 2018), and few account for autonomic activity. Chen et al (2020a) reported that vagal activity during SWS was a better predictor of WM improvement than SWA or vagal activity during wake (Chen, Whitehurst, et al., 2020a). In the current work, we found that changes in vagal autonomic activity during SWS, but not SWA per se, was critical. Together, the findings of vagal dominance during NREM sleep, close coupling between vagal activity and SWA, as well as the correlations between vagal activity during SWS and WM improvement suggest a non-negligible role of vagal influence on WM plasticity.

Given that both LTM and WM appear to rely on NREM sleep, many questions emerge. How are the limited resources of NREM sleep shared across memory processes? Is there a neural switch that toggles between central sigma-dependent LTM consolidation and autonomic vagal-dependent WM processing? What might be the sleep mechanisms supporting this ostensive neural switch?

Evidence has been shown of an antagonistic relation between neuromodulators governing the two systems, thalamocortical GABA and noradrenergic (NE) activity, respectively. Vagal nerve stimulation activates neurons in the locus coeruleus (LC) and increases NE levels in neocortex, hippocampus, amygdala, and other parts of the brain with afferent projections from LC (Hassert et al., 2004; Raedt et al., 2011; Roosevelt et al., 2006). Inactivation of LC significantly impairs working memory acquisition, while having no effect on consolidation or retention of spatial memories (Khakpour-Taleghani et al., 2009). In humans, increasing NE by transcutaneous-vagal-nerve-stimulation improves cognitive functions dependent on prefrontal networks, including WM and executive control, suggesting that LC-NE activity might promote cognitive control via more efficient neural processing (Chamberlain et al., 2006; Keute et al., 2020; Pihlaja et al., 2020; Sun et al., 2017).

Importantly, prior research has demonstrated mutual antagonistism between central sigma-dependent and autonomic vagal-dependent processes, with functional impact to cognition. Novitskaya and colleagues (2016) showed that LC-NE stimulation blocked the generation of

ripple-associated cortical sigma and caused spatial memory deficits, suggesting that with enough force, vagal-NE activity can inhibit hippocampal, sigma-dependent consolidation (Novitskaya et al., 2016). In addition, Beste and colleagues (2016) increase prefrontal NE levels in healthy human subjects using transcutaneous vagus nerve stimulation and showed increased inhibitory control during WM (Beste et al., 2016), whereas upregulating GABAergic networks impaired WM performance (Lozano-Soldevilla et al., 2014). On the other hand, using ripple-triggered fMRI in monkeys, Logothetis and colleagues demonstrated that ripples orchestrate a privileged state of enhanced central brain activity that may serve to boost communication between hippocampus and cortex by silencing output from the diencephalon, midbrain and brainstem (Logothetis et al., 2012), regions associated with autonomic regulation. In addition, in both humans and mice, Lecci et al. (2017) demonstrated that heart rate and sigma power oscillate in antiphase with each other at 0.02 Hz, suggesting a periodic switch between sigma and autonomic activation every 50 seconds (Lecci et al., 2017).

Here, using effective connectivity, we demonstrated that a GABAergic agonist enhanced naturally occurring cortical sigma dominance over vagal autonomic activity. Similar vagolytic findings have been shown with zolpidem in persistent vegetative state patients (Machado et al., 2014, 2011). Furthermore, the magnitude of this central influence predicted the trade-off between overnight LTM and WM improvement. Together with the previous literature, these finding suggest that central sigma-dependent processes, including GABAergic hippocampal-thalamocortical networks, and autonomic vagal-dependent processes, including noradrenergic frontal-autonomic networks, may compete for sleep resources during NREM sleep. We hypothesize that the shared resource may be the slow oscillation, which when coupled with sigma (and sharp-wave-ripples) promotes LTM, and when not coupled with sigma promotes WM. Given that approximately 20% of slow oscillations during NREM are sigma-coupled (Malerba et al., 2018), this leaves plenty of resources to be divided amongst other processes.

We propose a model for this trade-off in which the two memory processes (LTM and WM) alternate during NREM sleep via a complex interaction at the synaptic (GABA vs NE activation), systems (thalamocortical vs frontal-midbrain), and mechanistic level (sigma-coupled SO vs uncoupled SO) (see graphical model in Figure 5). Further research is required to tease apart these mechanisms and test their generalizability across multiple cognitive domains and tasks. The proposed sleep switch model may contribute insight to future translational research on cognitive disturbances observed in fronto-hippocampal neurodegenerative disorders such as Alzheimer’s disease and in fronto-striatal neurodegenerative disorders such as Parkinson's disease, both of which involve the degeneration of sleep (Mander et al., 2017; Targa et al., 2016).

Limitations

Limitations of this study include using a convenience sample of both men and women, and a lack of hormonal status among the young women, which can have an impact on cardiac vagal activity (see Schmalenberger et al., 2019 for a review) and sigma activity (Baker et al., 2019). Future studies examining hormonal fluctuation are needed to understand the interaction between central sigma and ANS profiles during sleep and their impact on cognition. Additionally, though we did not measure respiration directly, we did analyze the frequency peak of HF (HFfp)

in order to control for respiratory rate, which can affect the HRV (Song & Lehrer, 2003). HFfp showed no difference between the two drug conditions (see Supplemental Table S4 and S5) and varied within a narrow range in the HF spectrum, between 0.22 and 0.26 Hz. Thus, it is unlikely that respiratory activity played a key role in zolpidem's modulation on HRV and memory. However, we cannot completely exclude the effect of drug on cardiopulmonary coupling, as may be detected using measures of coherence (Thomas et al., 2005). In addition, our experimental design did not include an adaptation night, and thus may have caused the "first-night effect". However, the visits were counterbalanced by drug conditions, therefore the first-night effect should have canceled out across subjects. Lastly, due to methodological differences between EEG and ECG analyses, our results lack temporal specificity. Greater temporal precision around physiological events may provide insight into shifts between central- and autonomic-dependent activities.

Methods

Participants

34 adults in experiment 1 (M_{age} = 20.88 ± 1.88 years, 17 Females) and 38 adults in experiment 2 (M_{age} = 20.85 ± 2.97 years, 19 Females) with no history of neurological, psychological, or other chronic illnesses were recruited for the study (Supplemental Table S1 demographics). All participants signed informed consent, which was approved by the Western Institutional Review Board and the University of California, Riverside Human Research Review Board. Exclusion criteria included irregular sleep/wake cycles; sleep disorder; personal or familial history of diagnosed psychopathology; substance abuse/dependence; loss of consciousness greater than 2 minutes or a history of epilepsy; current use of psychotropic medications; and any cardiac or respiratory illness that may affect cerebral metabolism, which was determined during an in-person psychiatric assessment with trained research personnel. Additionally, all participants underwent a medical history and physical appointment with a staff physician to ensure their physical well-being. All subjects were naive to or had limited contact with (<2 lifetime use and no use in last year) the medication used in the study. Participants were asked to refrain from consuming caffeine, alcohol, and all stimulants for 24 h prior to and including the study day. Participants filled out sleep diaries for one week prior to each experiment and wore wrist-based activity monitors the night before the study (Actiwatch Spectrum, Philips Respironics, Bend, OR, USA) to ensure participants were well-rested (at least 7 hours per night during the week including the eve of the experimental day). Participants received monetary compensation and/or course credit for participating in the study. Study procedures were illustrated in Figure 1.

Data Reduction

Experiment 1

24 participants completed 4 visits (2 placebo nights and 2 zolpidem nights) and 8 participants completed 2 visits (1 placebo night and 1 zolpidem night). Therefore, 56 placebo and 56 zolpidem nights were included in the analyses.

Experiment 2

36 participants completed the placebo night and 35 participants completed the zolpidem night PSG recordings. 35 participants completed all three sessions of operation-span (working

memory) task in both placebo and zolpidem conditions. 33 participants completed all three sessions of word-paired associates (long-term memory) task in both placebo and zolpidem conditions.

Sleep Recording

EEG data were acquired using a 32-channel cap (EASEYCAP GmbH) with Ag/AgCI electrodes placed according to the international 10-20 System (Jasper, 1958). 22 electrodes were scalp recordings and the remaining electrodes were used for electrocardiogram (ECG), electromyogram (EMG), electrooculogram (EOG), ground, an online common reference channel (at FCz location, retained after re-referencing), and mastoid (A1 & A2) recordings. The EEG was recorded with a 1000 Hz sampling rate and was re-referenced to the contralateral mastoid (A1 & A2) post-recording. Data were pre-processed using BrainVision Analyzer 2.0 (BrainProducts, Munich Germany). Eight scalp electrodes (F3, F4, C3, C4, P3, P4, O1, O2), the EMG, and EOG were used in the scoring of the nighttime sleep data. High pass filters were set at .3 Hz and low pass filters at 35 Hz for EEG and EOG. Raw data were visually scored in 30-sec epochs into Wake, Stage 1, Stage 2, Slow Wave Sleep (SWS) and rapid eye movement (REM) sleep according to the Rechtschaffen & Kales' manual (Rechtschaffen & Kales, 1968) using HUME, a custom MATLAB toolbox. After staging, all epochs with artifacts and arousals were identified rejected by visual inspection before spectral analyses. Minutes in each sleep stage and sleep latencies (SL) (the number of minutes from lights out until the initial epoch of sleep, Stage 2, SWS and REM) were calculated. Additionally, wake after sleep onset (WASO) was calculated as total minutes awake after the initial epoch of sleep, and sleep efficiency (SE) was computed as total time spent asleep after lights out (~11:00PM) divided by the total time spent in bed (~11:00PM-9:00AM) * 100. Summary statistics for sleep architecture are shown in Table 3.

Power spectral analysis

The EEG power spectrum was computed using the Welch method (4 sec Hanning windows with 50 % overlap) (Campbell et al., 2005). SWA (0.5-2Hz), delta (1-4Hz), theta (4-8Hz), alpha (8-13Hz), sigma (12-16Hz), beta (15-30Hz), and total power (0.3-35Hz) were calculated for each sleep stage (Stage 2, SWS and REM). The EEG epochs that were contaminated by muscle and/or other artifacts were rejected using a simple out-of-bounds test (with a ±200 µV threshold) on high-pass filtered (0.5 Hz) version of the EEG signals. Then, the normalized power spectra (% power of each frequency band of interest/ total power) were averaged bilaterally within each sleep condition/stage/subject.

Heart Rate Variability

Electrocardiogram (ECG) data were acquired at a 1000-Hz sampling rate using a modified Lead II Einthoven configuration. We analyzed HRV of the R-waves series across the whole sleep/wake period using Kubios HRV Analysis Software 2.2 (Biosignal Analysis and Medical Imaging Group, University of Kuopio, Finland), according to the Task Force guidelines (Task Force, 1996). RR peaks were automatically detected by the Kubios software and visually examined by trained technicians. Incorrectly detected R-peaks were manually edited. Missing beats were corrected via cubic spline interpolation. Inter-beat intervals were computed, and a third-order polynomial filter was applied on the time series in order to remove trend components. Artifacts were removed using the automatic medium filter provided by the Kubios software.

The HRV analysis of the RR series was performed by using a Matlab-based algorithm (see Whitehurst et al., 2018). An autoregressive model (Model order set at 16; Boardman et al., 2002) was employed to calculate the absolute spectral power (ms2) in the LF HRV (0.04–0.15 Hz; ms2) and the HF HRV (0.15–0.40 Hz; ms2; an index of vagal tone) frequency bands, as well as total power (TP; ms2; reflecting total HRV), and HF peak frequency (HFpf; Hz; reflecting respiratory rate). From these variables, we derived the HF normalized units (HF_{nu} = $HF[ms^2]/HF[ms^2]+LF[ms^2]$) and the LF/HF ratio ($LF[ms^2]/HF[ms^2]$), an index often considered to reflect the sympathovagal balance (i.e., the balance between the two branches of the ANS), but whose meaning has been recently put into question (Billman, 2013; Reyes del Paso et al., 2013). The LF, HF, and TP measures had skewed distributions and as such were transformed by taking the natural logarithm, as suggested by Laborde et al., 2017. Since the LF normalized units are mathematically reciprocal to HF_{nu} (i.e. LF_{nu} =1- HF_{nu}), to avoid redundancy, only the HF_{nu} index is computed, an index often thought to reflect vagal modulation (Burr, 2007). Due to controversies about the physiological mechanisms that contribute to changes in LF activity, LF, LF/HF ratio and HFnu are difficult to make for these parameters, but they are reported for descriptive purposes.

In addition to the frequency domain parameters, RMSSD (ms; root mean square of successive differences) was calculated as a measure of vagally-mediated HRV in the time-domain. Similar to the frequency adjustments, to adjust for skewed distributions in the RMSSD, we report the natural logarithm. Additionally, RR (ms; time interval between consecutive R-peaks, reflecting frequency of myocardial contraction) were calculated as an index of cardiac autonomic control in our analyses (Pinna et al., 2007).

For time-domain and frequency-domain HRV measures during different sleep stages, consecutive artifact-free 5-min windows of undisturbed sleep were selected across the whole nap using the following rules: (a) the 1.5-min preceding, and (b) the entire 5-min epoch selected must be free from stage transitions, arousal, or movements. Windows were identified and averaged within Stage 2 sleep, slow-wave sleep (SWS), and REM sleep. We also analyzed 5 min of pre-sleep wakefulness (Rest). Epochs of N1 were not analyzed. Summary statistics for HRV variables are shown in Table 2.

Effective Connectivity

To explore the causal information flow between CNS and ANS sleep features, we considered sigma to reflect CNS activity and HFln to reflect ANS activity. Sigma power of eight EEG channels (F3, F4, C3, C4, P3, P4, O1, O2) and HF of HRV were considered as signals to estimate effective connectivity. To adopt uniform timing across signals and avoid temporal misalignments between EEG signals and HF time series, a sliding window technique was incorporated with window length of 5 minutes and stride of 5 seconds. All data during night time sleep was used to have continues time series of Sigma powers and HF, and length of 5 minutes was selected to be consist with HRV process. Therefore, for each subject, nine different signals were constructed including ratio of Sigma power band to total power of EEG of eight channels and HF power of HRV for each five-minute window (see Figure 3a).

Generalized partial direct coherence (GPDC) measure was used to estimate causal information flow between Sigma power and HF. GPDC uses multivariate vector autoregressive (MVAR) model to model causal interactions between signals and estimate directed causal information flow between signals by using the coefficients and parameters of MVAR.

After constructing Sigma power and HF signals, GPDC was computed for each window with length of 500 samples (2500 s) with stride of 250 samples. First, signals interactions were modeled by MVAR model (Eq. 1).

$$X(n) = \sum_{k=1}^{p} A_k X(n-k) + w(n) \tag{1}$$

Where $X(n)$ is the vector of signal values (with length of N, the number of signals, $N = 8$) in time n, $X(n) = [x_1(n), x_2(n), \dots, x_N(n)]^T$. p is order of the MVAR model which was selected according to Akaike criterion, $p = 4$. A_k is the matrix of MVAR coefficients and each element, $a_{ij}(k)$, stands how much j-th signal in time $n-k$ affects i-th signal in time n and $w(n)$ is the vector of model's additive Gaussian noise with zero mean and covariance matrix Σ. After modeling the interaction of the signals, GPDC was computed using frequency domain of coefficients and covariance matrix as:

$$\bar{\pi}_{ij}(f) = \frac{\frac{1}{\Sigma_{ii}} A_{ij}(f)}{\sqrt{\sum_{k=1}^{N} \frac{1}{\Sigma_{kk}{}^2} \left|A_{kj}(f)\right|^2}} \tag{2}$$

consequently:

$$0 \leq \left|\bar{\pi}_{ij}(f)\right|^2 \leq 1 \tag{3}$$

And

$$\sum_{i=1}^{N} \left|\bar{\pi}_{ij}(f)\right|^2 = 1 \tag{4}$$

$\bar{\pi}_{ij}(f)$ is the estimated matrix of causal information flow and the j-th column represent causal information outflow from the j-th signal to all the other signals. Average values over frequencies were considered for further process and based on the main purpose of the study two quantifier were defined as follow (see Figure 3a):

1. Causal information outflow from HF to all EEG channels, $HF_{Outflow}$ – Average (n=8) of causal information flow from HF to EEG sigma activity. $HF_{Outflow}$ represents the strength of causal effect of HF to Sigma power.
2. Causal information inflow to HF from all EEG channels, HF_{Inflow} – Average (n=8) of causal information flow from EEG sigma activity to HF. HF_{inflow} represents the strength of causal effect of Sigma to HF.
3. Effective connectivity ratio: HFInflow over HFOutflow, where greater numbers represented a greater central sigma control over autonomic vagal activity than vice versa.

Statistical Analyses

All statistical analyses were performed in R 3.6.2, using the libraries lme4 (Bates et al., 2015), and lsmeans (Lenth, 2016). P-values less than 0.05 were considered significant; p-values between 0.05 and 0.7 were considered trend-significant; p-values greater than 0.07 were considered non-significant. We used a linear mixed model (LMM) to evaluate the effects of zolpidem on sleep architecture, EEG power spectrum, autonomic profiles, and behavioral improvements. LMMs were chosen because it allows modeling of random effects and allow for the intercept and slope to be correlated (see Magezi, 2015 for detail explanation). LMMs are parametric models that use Maximum Likelihood Estimates (MLE) to obtain coefficients and

covariance structures. LMMs do not depend on limited assumptions about variance-covariance matrix assumptions (sphericity). Additionally, LMMs allow inclusion of an unbalanced number of observations per participants in the analyses. Moreover, LMMs models take into account the influence of factors whose levels are extracted randomly from a population (i.e. participants), thus yielding more generalizable results (Baayen et al., 2008).

Sleep architecture and Power spectrum

Using LMMs, we tested for the main effect of drug condition for sleep architecture (see Supplemental *Table S2 and S3)*, EEG power spectrum (see Supplemental *Table S6 and S7)*.

Autonomic Profiles

For autonomic profiles, we tested for the main effect of drug condition and interactions between sleep stage and drug condition by approximating likelihood ratio tests (LRT; Lewis et al., 2011) to compare LMMs with and without the effect of interest. We first built a reduced (nested) model, with sleep stage as the only effect, and then included drug condition as a fixed effect in the full model. By comparing the reduced and full model using the LRT, we can interpret if drug condition significantly modulated the outcomes. Tukey's correction for multiple testing was used for post-hoc comparisons.

Effective Connectivity

Using LMMs, we tested for the main effect of drug condition, the main effect of inflow vs outflow, and interaction between the two factors *(*see *Figure 3b and 3c)*. We first built a reduced (nested) model, with inflow vs outflow, as the only effect, and then included drug condition as a fixed effect in the full model. By comparing the reduced and full model using the LRT, we can interpret if drug condition significantly modulated the outcomes. Tukey's correction for multiple testing was used for post-hoc comparisons.

Behavioral Tasks

To investigate the drug effect on cognitive enhancement, LMMs were used with the drug condition as the predictor of interest (fixed effect), the improvement in WPA and OS tasks as outcome variables, and participants as crossed random effects. As we assume larger individual differences of improvement and difference in improvement between drug conditions, our LMMs include both a random intercept and a random slope term. To account for practicing effect on the tasks, we included visit and baseline performance as a covariate in the models. We first confirmed no differences at baseline (Test 1) between the placebo and zolpidem visits (*see Supplemental Figure S1*). Next, we confirmed no differences of improvements across 12-hr of waking (Test 2 – Test 1) between the placebo and zolpidem visits (*see Supplemental Figure S1*). We then tested the sleep-dependent changes in improvement: the overnight (Test 3 – Test 2) and 24-hr (Test 3 – Test 1) changes (see Figure 4a and 4b). Again, we tested for the effect of drug condition by approximating LRTs.

Correlations

Lastly, we used a Pearson's correlation coefficients to examine the functional roles of sigma, vagal activity, and causal information flow on sleep-dependent behavioral changes.

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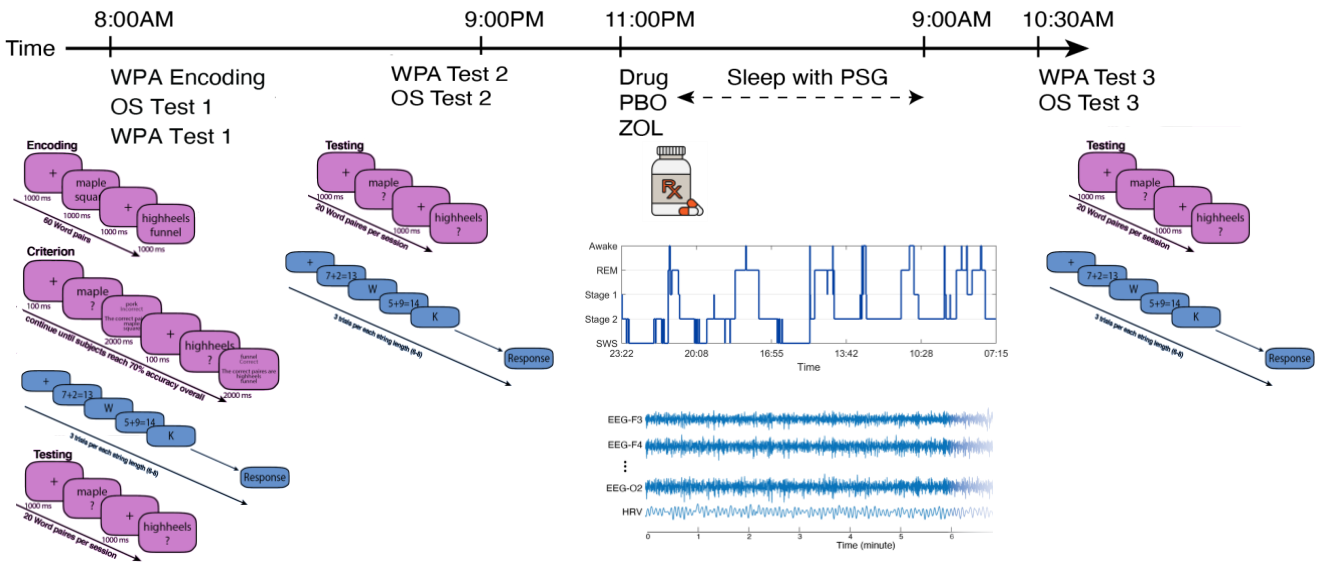


Figure 1. Experimental design and behavioral tasks.

Experiment 1*: Participants reported to the lab at 9:00PM and were hooked up to polysomnography (PSG), including electroencephalography (EEG), electrocardiogram (ECG), electromyogram (EMG), and electrooculogram (EOG). Before sleep, we recorded 5-min resting HRV while subjects lay awake in a still, supine position. At 11:00PM, directly before lights-out, subjects ingested either 10mg of zolpidem or placebo. Sleep was monitored online by a trained sleep technician. Participants were woken up at 9:00AM the next morning and permitted to leave the lab. Each participant experienced two visits per drug condition (a total of four visits).*

Experiment 2*: At 8:00AM, participants began encoding for the episodic memory word-paired-associates (WPA) task, followed by the working memory operation-span task (OS) task and immediate recall for the WPA (Test 1). Participants left the lab after cognitive testing. Participants were asked not to nap, exercise, or consume caffeine or alcohol, and were monitored with actigraphy during the break. Participants returned to the laboratory at 9:00 PM to complete the delayed recall over wake for WPA and OS (Test 2). Participants were then hooked up to polysomnography (PSG), including electroencephalography (EEG), electrocardiogram (ECG), electromyogram (EMG), and electrooculogram (EOG). Before sleep, we recorded 5-min resting HRV while subjects lay awake in a still, supine position. At 11:00PM, directly before lights-out, subjects ingested either 10mg of zolpidem or placebo. Sleep was monitored online by a trained sleep technician. Participants were woken up at 9:00AM the next morning and provided a standardized breakfast. At 10:30 AM, participants completed the delayed recall over sleep for WPA and OS (Test 3). For both tasks, to assess the change in performance, we measured two difference scores: overnight change (Test 3 – Test 2); 24-hr change (Test 3 – Test 1). Each participant experienced one visit per drug condition (a total of two visits). See Supplemental Figure S1 and Table S8 for summary statistics.*

Word-paired associates (WPA) task*: Subjects were visually presented with unrelated word pairs (e.g. maple-square) to maximize novel associations. Words were 3–9 letters in length and drawn*

from a normative set of English nouns. The WPA task consisted of an encoding phase and three recall phases. Encoding consisted viewing 60 pairs of words, each presented vertically stacked and shown twice in random order. Every word pair was presented for 1000ms followed by a fixation cross for 1000ms. Immediate after the encoding, subjects are trained to criterion using a test in which participants were shown one word of the pair and were required to type in the associated word. Feedback was provided and participants had to achieve 70% accuracy to finish the training. Incorrect trials were repeated after a variable interval. Recall tests were assessed at immediate recall (9AM, Test 1), over wake (9PM, Test 2), and over sleep (10:30AM, Test 3). For testing, the 60 word-pairs were divided into three sets of 20 pairs, with one set tested at each test session and the order counterbalanced. To ensure that the subjects were not exposed to repeated stimulus, different versions of word lists for each visit were imposed.

***Operation Span (OS) task**: Participants performed mental arithmetic while memorizing a set of unrelated letters. The task included 3 practice and 9 test trials. Participants were tested in letter strings six to eight. For each letter string, participants were shown a series of math problems that they had to confirm were correct within 3 seconds, using pre-determined responses on the keyboard. After each equation, a letter would appear on the screen and the subject was instructed to remember each letter. At the end of each string, the participant was instructed to recall the letters in the order presented by typing responses on a computer keyboard. Immediately after each trial, the next letter string would be presented. An example of a six-item trial might be: 12 - 2 = 8 (correct/ incorrect?) => J; 6 + 7 = 14 (correct/incorrect?) => G; 3 - 2 = 1 (correct/incorrect?) => S; 5 + 7 = 13 (correct/incorrect?) => K; 8 + 7 = 14 (correct/incorrect?) => H; 5 + 9 = 14 (correct/incorrect?) => L. After verifying the six equations in this example, participants were asked to type the presented letters in the order that they were presented (in this case JGSKHL). If the participants forgot one of the letters in a trial, they were instructed to provide their best guess. In addition, to decrease trade-off between solving the operations and remembering the letters, an 85% accuracy criterion on the math operations was required for all the participants. Based on this criterion, four participants during the ZOL condition were excluded. We calculated performance as: number of correct letters recalled in the correct position divided by total number of letters in the string per trial, and then averaged over the total trials.*

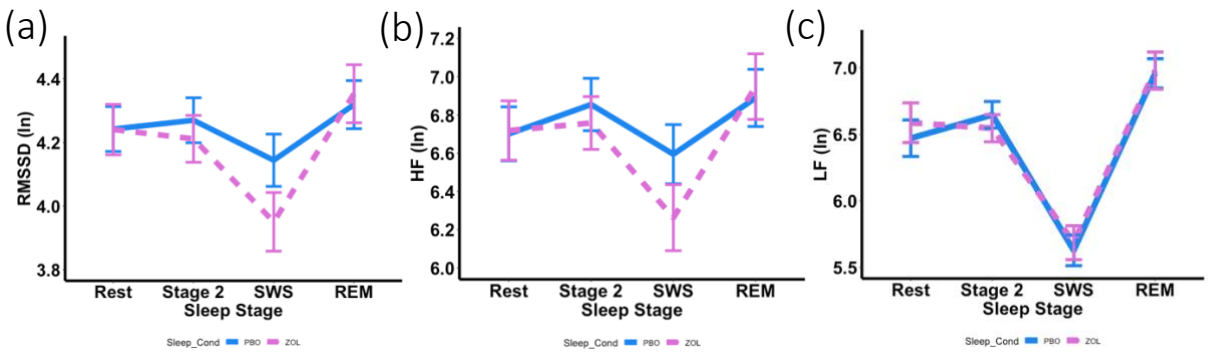


Figure 2: Zolpidem decreased vagal HF HRV, but not LF, during SWS.

(a) For RMSSDln, we report a significant main effect of sleep stage ($F(3, 366) = 21.257$, $p < .0001$), with a decreased HRV during SWS compared to Rest, Stage 2, and REM (all ps < .0001). We also found a significant interaction ($F(3, 366) = 3.8630$, $p = .0096$) between sleep stage and drug condition, with decreased vagal activity during SWS ($p = .0006$) in zolpidem compared with placebo, but not during Stage 2 ($p = .3549$), REM ($p = .3804$), or Rest ($p = .6152$). The likelihood ratio test was significant (LR = 13.8544; $p = .0078$), suggesting that zolpidem significantly modulated the time-domain measure of HRV.

(b) For HFln, we report a significant main effect of sleep stage ($F(3, 366) = 16.9891$, $p < .0001$), with a decreased HRV during SWS compared to Rest ($p = .0006$), Stage 2 ($p < .0001$), and REM ($p < .0001$). Similarly, we also report a significant interaction ($F(3, 366) = 3.1899$, $p = .0238$) between sleep stage and drug condition, with decreased vagal activity during SWS ($p = .0020$) in zolpidem compared with placebo, but not during Stage 2 ($p = .4194$), REM ($p = .4365$), or Rest ($p = .6070$). The likelihood ratio test was significant (LR = 11.3671; $p = .0227$), suggesting that zolpidem significantly modulated the frequency-domain measure of HRV.

(c) For LFln, we report a significant main effect of sleep stage ($F(3, 366) = 93.0330$, $p < .0001$), with a decreased LF power during SWS compared to Rest, Stage 2, and REM (all ps < .0001), and an increased LF power during REM compared to Rest and Stage 2 (all ps < .0001. No significant main effect of drug condition ($p = .6337$), nor interaction between sleep stage and drug condition ($p = .5681$) were found. The likelihood ratio test was not significant (LR = 2.2889; $p = .6828$), suggesting that zolpidem did not significantly modulate low frequency HRV.

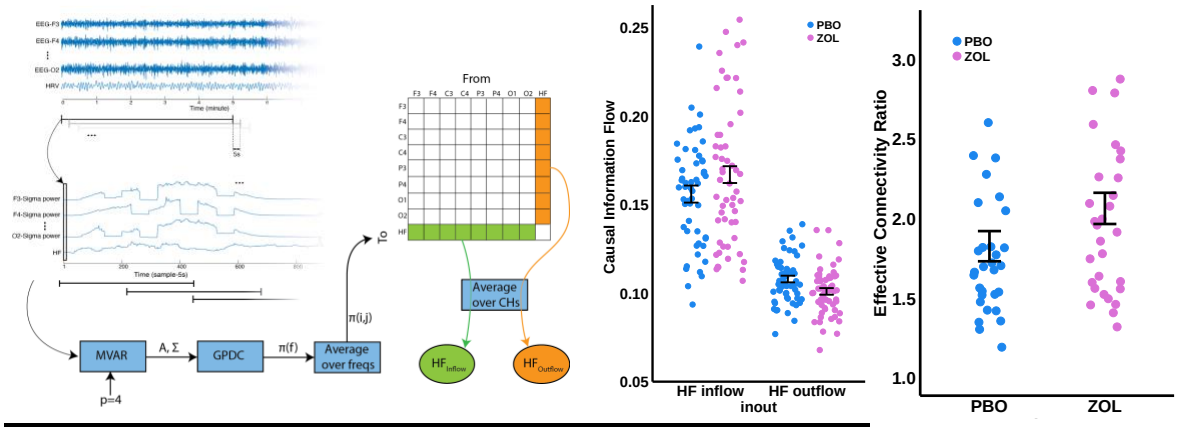


Figure 3: Effective Connectivity

(a) Effective Connectivity Estimation Procedure

(b) Experiment 1: We report a main effect of inflow vs outflow ($F(1, 185) = 273.317$, $p < .0001$), with a greater HFinflow than HFoutflow in both drug conditions; an interaction between drug condition and inflow vs outflow ($F(1, 185) = 5.744$, $p = .0175$), with a greater HFinlfow during zolpidem compared to placebo ($p = .0369$). No main effect of drug condition was found ($F(1, 185) = 0.512$, $p = .4751$). The likelihood ratio test was significant ($LR = 6.0745$; $p = .0480$), suggesting that zolpidem significantly modulated the causal information flow between sigma and HF activity. Effective connectivity ratios (HFInflow/ HFOutflow) increased significantly during the zolpidem night ($F(1, 79) = 8.0607$, $p = .0059$).

(c) Experiment 2: Effective connectivity ratios (HFInflow/ HFOutflow) increased significantly during the zolpidem night ($F(1, 32) = 5.4087$, $p = .0265$).

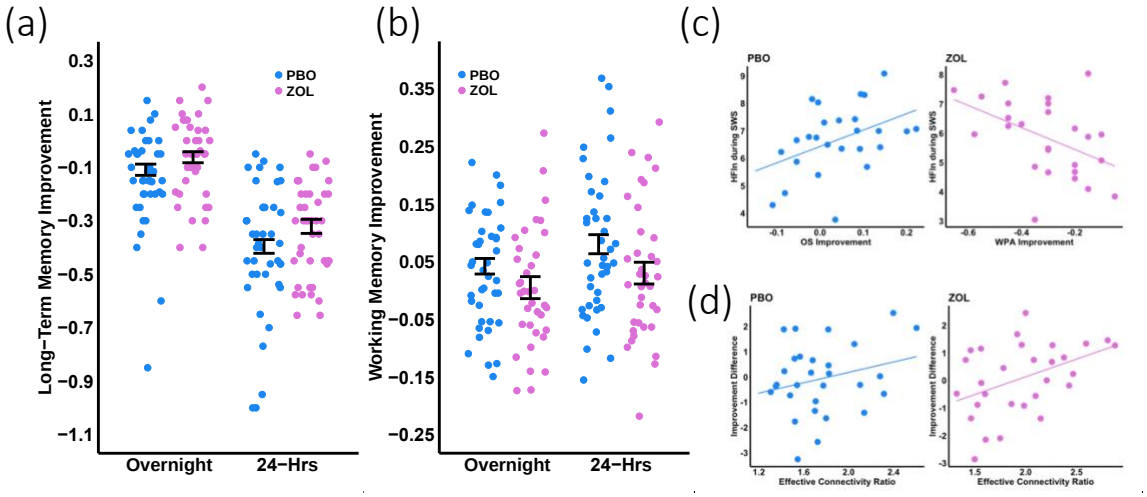


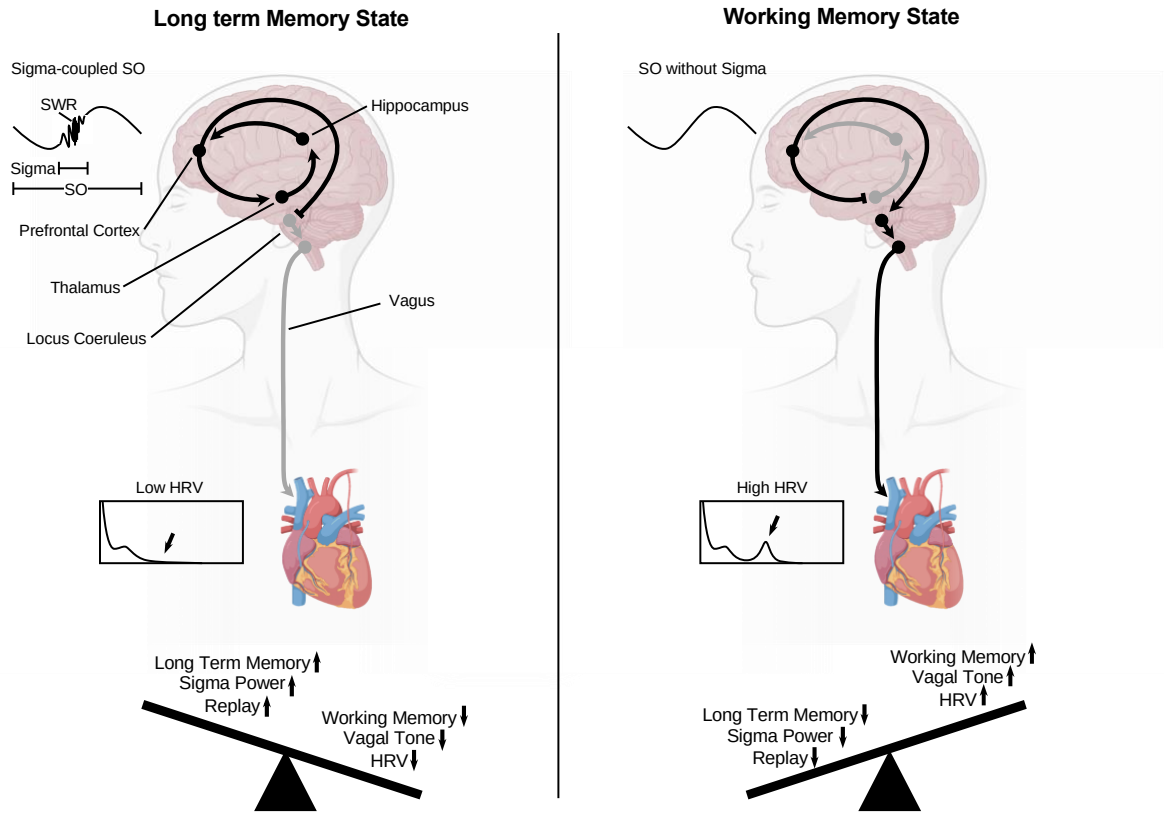
Figure 4: Zolpidem increases LTM, but decreases WM improvement

(a) Long-term memory (WPA task) improvement by drug conditions and time. *(Y axis: WPA Test3-Test2 and Test3-Test1; asterisks indicate significant differences in behavioral changes between two drug conditions; *p<0.05) ZOL yielded greater but not significant overnight retention of WPA than the PBO visits (estimate= -0.1156, CI= (-0.2408, -0.0095), t= -1.8104, p= 0.0810), accounting for visit, as well as greater 24-hr retention of WPA than PBO visits (estimate= -0.1810, CI= (-0.3519, -0.0096), t= -2.0704, p= 0.0474), accounting for visit.*

(b) Working memory (OS task) improvement by drug conditions and time. *(Y axis: OS Test3-Test2 and Test3-Test1; asterisks indicate significant differences in behavioral changes between two drug conditions; *p<0.05) PBO showed significantly greater overnight improvement of OS than ZOL visits (estimate= 0.1242, CI= (0.0201, 0.2284), t= 2.3377, p= 0.0260), accounting for Test 2 performance and visit, as well as greater but not significant 24-hr improvement of OS than ZOL visits (estimate=0.1000, CI= (-0.0184, 0.2185), t= 1.6546, p= 0.1081), accounting for Test 1 performance and visit.*

(c) Functional role of vagal activity on memory. *(Y axis: HFln during SWS, X axis: OS Test3-Test2 and WPA Test3-Test1) Vagal activity during SWS positively predicted working memory (OS task) improvement (r = .422; p = .032) but negatively predicted long-term memory (WPA task) improvement (r = -.460; p = .018).*

(d) Functional role of effective connectivity ratio on memory trade-off. *(Y axis: normalized WPA improvement - OS improvement score, X axis: effective connectivity ratio = HFInflow/ HFOutflow) Effective connectivity ratio (a higher ratio indicates a greater causal effect from sigma to vagal) during sleep positively predicted memory trade-off (a greater difference indicates a greater improvement in the WPA task than the OS task) during the zolpidem night (r = .429; p = .020), but not the placebo night (r = .251; p = .190).*



	Long Term memory	Working memory
Brain Regions	Thalamus, Hippocampus, Prefrontal cortex	Prefrontal cortex, Locus Coeruleus
Neuromodulators	GABA	Norepinephrine
Sleep Mechanisms	Coupling between Sharp Wave Ripple (SWR)-Sigma-Slow Oscillations (SO) reflects LTM systems consolidation.	Uncoupled SO during SWS reflects Prefrontal Cortex inhibitory network activation and greater autonomic vagal HRV.

Figure 5: Sleep Switch Model

The model represents the proposed brain regions, primary neuromodulators, and sleep mechanisms involved in the Long-term memory state and the Working memory state that toggle throughout non-rapid eye movement (NREM) sleep. During the Long-Term Memory state, consolidation occurs via sigma-coupled SOs, which leads to reduced autonomic vagal-dependent activity and less WM improvement. During the Working Memory state, greater efficiency occurs during uncoupled SOs associated with increased autonomic vagal-dependent activity, which leads to reduced central sigma-dependent activity and less LTM consolidation.