Specific changes in sleep oscillations after blocking human metabotropic glutamate receptor 5 in the absence of altered memory function

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Abstract

Sleep consolidates declarative memory by the repeated replay of neuronal traces encoded during prior wakefulness. This replay during sleep is linked to the cardinal oscillations of NonREM sleep. Although this replay is ideally suited to support plasticity, there is so far little evidence of classical glutamatergic plasticity playing a role in this process. To the contrary, we have previously reported that blocking NMDA or AMPA receptors does not affect sleep-dependent consolidation of declarative memory. Here, in two placebo-controlled within-subject cross-over experiments with 20 healthy humans each, we used fenobam to block metabotropic glutamate receptor 5 (mGluR5) during sleep. In Experiment I, participants learned word-pairs (declarative task) and a finger sequence (procedural task) during the evening before being administered the treatment and then sleeping for 8 hours – recall was tested in the next morning. To cover possible effects on synaptic renormalization processes during sleep, in Experiment II, participants learned new word-pairs in the morning after sleep. Surprisingly, fenobam neither reduced retention of memory across sleep nor new learning after sleep, although it severely altered sleep architecture and memory-relevant EEG oscillations. In NonREM sleep, fenobam suppressed 12-15 Hz spindles but augmented 2-4 Hz delta waves, whereas in REM sleep it suppressed 4-8 Hz theta and 16-22 Hz beta waves. Notably, under Fenobam NonREM spindles became more consistently phase-coupled to the slow oscillation. Our findings indicates that mGluR5-related plasticity is not essential for memory processing during sleep, even though mGlurR5 are strongly implicated in the regulation of the cardinal sleep oscillations.

Introduction

The memory enhancing function of sleep has been reported time and again (for a comprehensive review see: [1,2], for current developments see: [3,4], however also see [5]). Sleep's role for memory relies on the repeated replay of memory traces that were encoded during prior wakefulness [6] and has been linked to the systems consolidation of hippocampus-dependent declarative memory [7,8]. The hippocampo-neocortical dialog mediating this systems consolidation process is supported by the intricate interplay between slow oscillations (~0.75 Hz), sleep spindles (12-15 Hz) and sharp-wave/ripples (~100 Hz) that are observed during non-rapid-eye-movement (NonREM) sleep [9,10]. While information flows from the sensory cortices to the hippocampus during wake, the direction of information flow is reversed during NonREM sleep [11] and this reversal relies on low levels of acetylcholine during early slow wave sleep (SWS) rich NonREM sleep [12,13]. Hippocampal replay as well as output from these neuron assemblies towards the neocortex is principally conveyed via glutamatergic neurons. Moreover, most researchers agree that replay and the associated neuronal oscillations are ideally suited to support systems consolidation by eliciting Hebbian plasticity in local synaptic circuitry [14,15]. Nevertheless, so far, our attempts to link sleep-dependent declarative memory consolidation to classical glutamatergic processes have largely failed [16]. Here, we present our findings in pursuit of ascertaining the role of less well-characterized glutamatergic processes on sleep-dependent memory processes.

Classical glutamatergic N-methyl-D-aspartate (NMDA) receptor dependent long-term-potentiation (LTP) represents the most thoroughly investigated and, thus, prototypic form of neuronal plasticity in the mammalian brain [17,18]. In brief, an incoming action potential elicits an excitatory post-synaptic potential at the post-synaptic neuron via sodium influx through the ion channel associated to the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor. The NMDA receptor acts as a coincidence detector, inasmuch as, it is blocked by magnesium until the post-synaptic neuron is sufficiently depolarized by several incoming action potentials arriving within a certain time or space. In such a case, glutamate binding to the

NMDA receptor allows the influx of calcium, finally, eliciting classical LTP, e.g., by enhancing signal transduction through AMPA receptor phosphorylation. In line with their essential role for brain plasticity, we could show that blocking NMDA or AMPA receptors during human sleep with ketamine or caroverine, respectively, completely abolished sleep-dependent performance gains for cortical memory [19]. In contrast, applying the very same drugs during sleep did not impair the retention of declarative word pairs that relies on hippocampal areas for initial storage [16], although, the sleep-dependent consolidation of word-pairs was enhanced by the NMDA receptor co-agonist d-cycloserine.

The metabotropic glutamate receptor 5 (mGluR5) can be found throughout the hippocampus [20] and has been shown to contribute to NMDA receptor mediated LTP in the hippocampus [21-23]. In humans, levels of mGluR5 were increased after sleep-deprivation [24]. A functional shift from the NMDA receptor to mGluR5 mediating enhanced calcium influx in the potentiated synapse offers a potential explanation for the above-mentioned negative findings after blocking of NMDA receptors during human sleep. In fact, activating mGluR5 has even been directly shown to reverse NMDA receptor blockade by ketamine [25].

mGluR5 has also been found to contribute to long-term-depression (LTD, [22,26]) – the opposite of LTP, inasmuch as, LTD reduces synaptic transmission by, e.g., de-phosphorylating AMPA receptors. This is of importance here because the replay-based systems consolidation process may indeed upscale cortical synapses to enhance a memory, but ripples may simultaneously weaken and downscale its hippocampal synapses [27]. In addition, sleep exerts a global renormalizing effect on synaptic weights thereby erasing irrelevant memory traces and ensuring that the brain retains sustainability [3,28] which explains why the ability to learn new information is enhanced after sleep [29,30].

In the current research, we applied fenobam, a selective blocker of mGluR5 [31], during retention sleep in healthy humans. To investigate effects on sleep-dependent consolidation. In Experiment 1, participants learned word-pairs during the evening and then slept after receiving treatment. Retrieval was tested the next day. To additionally cover possible effects of fenobam

on processes of synaptic downscaling and renormalisation, in Experiment 2, participants also received the drug before sleep after learning word-pairs. However, on the next morning their capability of learning new word-pairs was assessed. We expected that blockade of mGluR5 impairs sleep-dependent consolidation of the word-pairs as well as sleep-dependent improvements of new learning.

Methods

Participants

Forty participants completed the current study (n = 20 for each experiment). Participants were healthy, non-smoking, native German-speaking men (18–30 years), who reported no chronic physical or psychological illness in present or past. Participants underwent a routine health examination prior to participation to exclude any mental or physical disease, did not take any medication at the time of the experiments, and reported a normal sleep–wake cycle. The participants were instructed to get up at 07:00 on experimental days, and during these days not to take any naps and not to ingest alcohol or (after 13:00) caffeine-containing drinks. Before the experiment proper, participants took part in an adaption night under conditions of the experiment (i.e., including the placement of electrodes for polysomnographic recordings).The experiments were approved by the local ethics committee (Ethics Committee of the Medical Faculty at the University of Tübingen). Written informed consent was obtained from all participants before participation. Participation was compensated financially.

Design and Procedures

Both experiments followed a double-blind, placebo-controlled, within-subject, balanced crossover design (see Figure 1A for an overview). Within each experiment, each participant underwent two identical sessions with the exception of receiving either fenobam or placebo, separated by at least 14 days. In the 1980s, fenobam was developed as a novel non-benzodiazepine anxiolytic, but research was discontinued due to apparent neurological side effects [32]. We chose fenobam due to its selective mGluR 5 blocking properties [31] and it was synthesised according to Good Laboratory Practice by Syncom (Groningen, Netherlands). Identical looking placebo and verum capsules were produced according to Good Manufacturing Practices by the Department of Pharmacy & Pharmacology at Slotervaart Hospital (Amsterdam, Netherlands). Each verum capsule contained 365 mg fenobam (plasma peak: 180 min, plasma half-life: 120 min, rough estimates according to [33]), i.e., the human equivalent dose of the dose that effectively blocked encoding in rats [34].

Experiment 1 – Participants arrived at the lab at 20:00 and were prepared for polysomnography. Next, they learned word-pairs and a finger sequence tapping task. Afterwards, vigilance, mood, adverse side effects and sleepiness were assessed and at 22:00 they received a capsule containing either fenobam or placebo. They watched animal documentaries until the electrodes were connected to the amplifier and at 23:00 the light was turned off. Participants were woken after 7:45-8:15 hours, preferentially from sleep stages 1 or 2 and sleepiness, adverse side effects as well as mood were assessed. Next, they were allowed to take a shower, they received a standardised breakfast and again watched animal documentaries to pass the time. At 10:00 retrieval was tested and additional control tasks were performed. Finally, vigilance, mood, adverse side effects and sleepiness as well as general retrieval performance were assessed.

Experiment 2 – The timeline was identical to Experiment 1. However, we used a different protocol for word-pair learning in the evening and introduced learning of new word-pairs at 10:00 the next morning. In this experiment we did not assess finger sequence tapping.

Memory Tasks

Word-pairs - The word-pair memory task was used to test hippocampus-dependent declarative memory. We decided to use more word-pairs than previously [16] to avoid ceiling effects and thus optimize the expected effect size [35]. In Experiment 1, participants were presented with 120 word-pairs (in three blocks of 40 word-pairs with a short break between each block) for 4 s each and with an inter stimulus interval (ISI) of 1 second during the Learning phase in the evening (procedure adapted from [36]). Immediate recall was tested afterwards in a cued recall procedure, i.e., participants were shown the first word and had to respond by saying the second word. Irrespective of their performance they were shown the correct word as feedback for 2 s. In the next morning, during the Retrieval phase, delayed recall was tested using the same procedure but without feedback. Absolute differences between word pairs recalled during the Retrieval phase and during the Learning phase served as a measure of overnight retention.

In Experiment 2, participants were presented with 80 word-pairs (A-B in two blocks of 40) for 4 s each and with an ISI of 1 second during learning (procedure adapted from [16] and [37]). Immediate recall was tested using the same cued recall procedure as in Experiment 1. However, participants received no direct feedback and learning (3 s per stimulus) and immediate recall were repeated, if performance was below 60 %. During the New Learning phase before retrieval, participants learned 80 new word-pairs, half of which interfered with the original (A-B) word-pairs inasmuch as they shared the cue word (A-C), and half of which were entirely new (D-E). Word-pairs were learned in three blocks of learning followed by immediate cued recall. Word-pairs were presented for 4 s each during initial learning in the first block and for 3 s in the following block. Absolute values of word-pairs were used as a measure of new learning after sleep. During the subsequent Retrieval phase, recall of the original A-B word-pairs was tested again. Absolute differences between word-pairs recalled during the Retrieval phase and on the criterion trial during the Learning phase before sleep served as a measure of overnight retention.

Finger sequence tapping - In Experiment 1, the finger sequence tapping task was used to test procedural memory that is not critically depending on hippocampal function and has been described previously for having strong sleep-dependent effects [38,39]. Essentially, participants used their non-dominant hand to press a finger sequence (e.g., 4-1-3-2-4) on a keyboard as fast and as accurately as possible during twelve 30-second blocks interrupted by 30-second breaks. The numeric sequence was displayed on the screen at all times to keep working memory demands at a minimum. A key press resulted in a white asterisk appearing underneath the current element of the sequence. Each 30-second block was scored for speed (number of correctly completed sequences) and errors. After each 30-second block, feedback was given about the number of correctly completed sequences and the error rate. During the Learning phase, participant trained on twelve 30-second blocks. The average score for the last three of these trials was used to indicate learning performance. During the Retrieval phase the next morning, participants were tested on another three blocks. The absolute difference

between these was used as a score of retention performance. Afterwards, as a control for non-specific effects on learning a new sequence was learned for an additional 12 blocks.

Polysomnography and Sleep Stage Analyses

The polysomnography was performed using Ag-AgCl electrodes connected to a Brain Amp DC 32-channel EEG-amplifier (Brain Products GmbH, Gilching) for the EEG channels and a Brain Amp ExG 8-channel bipolar amplifier (Brain Products GmbH, Gilching, Germany) that shared a ground connected to the forehead. The EEG was recorded from F3, Fz, F4, C3, Cz, C4, P3, Pz, P4 according to the international 10-20 system and referenced to coupled electrodes attached to the mastoids. Additionally, horizontal and vertical eye movements and muscle tone (from the chin) were recorded for standard polysomnography. The data were low-pass (80 Hz) and high-pass filtered (10 s time-constant) and sampled at 250 Hz. For sleep scoring the data were down-sampled to 200 Hz and additional offline low-pass (35 Hz) and high-pass (5 s time-constant) zero-phase Butterworth filters were applied. Sleep stage were scored according to Rechtschaffen and Kales [40], using C3 and C4, by two experienced investigators who were blind to the assigned treatment Differences in scoring between the scorers were resolved by consulting a third experienced investigator.

EEG analyses

During sleep scoring, it was evident that the drug influenced sleep EEG patterns severely enough to be identifiable by the naked eye during blind scoring. Therefore, in a subset of 24 participants (N = 11 from Experiment 1 and N = 13 from Experiment 2), for whom EEG signals in all 9 channels were of sufficient quality, more detailed EEG analysis were performed in MATLAB (v2016a, MathWorks, Natick, MA) using FieldTrip [41] and CircStat [42] toolboxes as well as by applying custom MATLAB functions (see details in the sections below). Firstly, normalized, 1/f corrected power spectra during NonREM and REM sleep were compared between fenobam and placebo. Secondly, distinct oscillatory events were detected and characterized regarding their density, amplitude, and frequency. Finally, the phase-amplitude

coupling (PAC) between slow oscillations (SO) and spindles was assessed, because of its known relevance for memory consolidation during sleep.

Power spectral analysis

Power spectra were calculated using a Hanning-tapered Fast Fourier Transform (FFT) of non-overlapping artefact-free 4 s epochs (with a resolution of 0.25 Hz between 0.25 and 25 Hz), separately for NonREM and REM sleep, using the method of Irregular-Resampling Auto-Spectral Analysis (IRASA) [43], which has recently been used to successfully remove the 1/f noise from EEG power spectra obtained during sleep [44]. In brief, IRASA resamples the time domain EEG data for each epoch using pairwise symmetric non-integer resampling factors (here: 1.1-1.9 in steps of 0.05 and corresponding factors 0.9-0.1) before calculating the FFT, resulting in multiple power spectra per epoch with artificially shifted peak frequencies for all oscillatory signals, while the 1/f power law noise component remains unchanged by resampling due to its fractal nature. Median-averaging of all power spectra per epoch then removes any frequency-shifted oscillatory signals and preserves only the fractal 1/f component, which in turn can be subtracted from the original power spectrum (of the non-resampled data) to obtain the 1/f-corrected power spectrum. We eventually calculated a normalized version of the 1/f-corrected power spectra by expressing the power in each frequency bin as the percentage change from the respective fractal power at that frequency. This approach elegantly normalizes oscillatory power per subject, channel, and frequency, thereby not only uncovering less pronounced oscillations that are usually hidden in 1/f noise, but also considerably reducing the large inter-subject variability in oscillatory power.

Event detection analysis

To complement spectral power analyses, we detected and characterized discrete oscillatory events using detection algorithms established for SOs and spindles in NonREM sleep [9,10,45]. We used the latter in adapted form also to detect transient bursts of delta and theta oscillatory activity during NonREM sleep as well as theta, alpha, and beta oscillatory activity during REM sleep. Importantly, amplitude criteria for event detection were determined

individually per night and channel. While a common absolute threshold or a common relative threshold across all nights enables confounding of oscillatory event detection by average oscillatory band power, relative thresholds determined per night control for potential power changes when analysing the density of discrete oscillatory events. In general, only artefact-free data from NonREM sleep stages S2-S4 or REM sleep were used for event detection, respectively, and data were always 50 Hz notch filtered to remove line noise. All bandpass-filters (see below) were zero-phase (two-pass) FIR filters with an order of 3 cycles of the respective low frequency cut-off.

For identifying SO events, data were bandpass-filtered 0.16-1.25 Hz, all zero-crossings were determined, and event duration of single waves was determined as time between two successive positive-to-negative zero-crossings. Events meeting the SO duration criteria (0.8-2 s corresponding to 0.5-1.25 Hz) were kept and their peak-to-peak amplitudes extracted. Events also meeting the SO amplitude criteria (greater than mean + 2 SD of the distribution of all extracted amplitudes) were then considered SOs, and epochs (-2.5 to +2.5 s) time-locked to the SO down-state negative peak in the filtered signal were extracted from the unfiltered raw signal for all events. For spindle detection, data were bandpass-filtered 12-15 Hz, and the root mean square (RMS) signal was calculated using a moving average of 200 ms. Whenever the signal exceeded the threshold of mean + 2 SD of the RMS signal (spindle amplitude criterion) for 0.5-3 s (spindle duration criterion) a spindle event was detected, and epochs (-2.5 to +2.5 s) time-locked to the maximum spindle trough in the filtered signal were extracted from the unfiltered raw signal for all events.

For the other oscillatory events, i.e., NonREM delta and theta as well as REM theta, alpha, and beta oscillatory events, detection procedures were analogous to the spindle detection pipeline, but with adapted bandpass-filter limits (delta: 2-4 Hz, theta: 4-8 Hz, alpha: 8-12 Hz, beta: 16-22 Hz) and event duration criteria (lower duration limit: 3 cycles of the centre frequency of the respective oscillatory band, e.g., for theta (2-4 Hz), at least 3 cycles at 6 Hz = 0.5 s; and no upper duration limit). Per subject, event density was calculated as number of

events per minute, and average event amplitude was based on the respective maximal peak-to-peak amplitude of the filtered signal within the event limits. Individual peak frequency was derived from the normalized 1/f corrected power spectra as power maximum within the respective frequency band limits.

SO-spindle phase-amplitude coupling (PAC) analysis

To assess the relationship between SO phase and spindle power we analysed SO-spindle phase-amplitude coupling (SO-spindle-PAC) as described in [9]. Both the phase of the SO bandpass-filtered signal and the phase of the spindle bandpass-filtered spindle power signal over time were extracted using the Hilbert transform. The spindle power modulation over time was estimated via a Hanning-tapered windowed FFT (FieldTrip's 'mtmconvol' function) for frequencies between 12 and 15 Hz in steps of 1 Hz and a sliding window of 5 times the respective cycle length of a given frequency, moving in 10 ms steps. Frequency bins within the spindle range were averaged per time bin and upsampled to the EEG sampling rate of 250 Hz to align them with the SO phase estimates. The synchronization index between the two phase value time series [46,47], was then calculated for each SO epoch -1 to +1 s around the SO down-state. Averaging of phase-delays across SO events (CircStat toolbox) then provided for each participant the average SO phase angle at which spindle power was maximal (Figure 3D), as well as the consistency of this phase relationship across events, reflected by the respective mean vector length (Figure 3D). The deviation of individual SO-spindle-PAC phases from the group average indicated the consistency across participants (Figure 3E).

Control Measures—Vigilance, Sleepiness, Mood Ratings, Word Fluency, Picture and Number Learning

Sleepiness and mood were assessed in the evening during the Learning phase as well as the next morning after awakening and during the Retrieval phase. Sleepiness was measured using the Stanford sleepiness scale (SSS; [48]). Mood was assessed using the multidimensional mood questionnaire (MDBF; [49]). Additionally, during the Learning phase and the Retrieval phase vigilance was tested using a five minute version of the psychomotor vigilance task (PVT;

[50]) that required pressing a button as fast as possible whenever a bright millisecond clock, present on a dark computer screen, started counting upward. After the button press, this clock displayed the reaction time. Adverse side effects were measured by providing the most often encountered side effects under fenobam and offering an answer on a five point scale ranging from ‘not at all’ to ‘very much’ as well as a free text field.

Word fluency [51] was tested at retrieval to control for effects on recall of long-term memories that have already been strongly consolidated. Participants produced as many words as possible prompted by a letter (p or m) and a category (hobby or occupation) cue for two minutes each. The sum of both versions was used a measure of general retrieval performance.

We additionally used a picture learning task and a number learning task at retrieval as non-verbal memory control tasks. In the picture learning task (that was only used in Experiment 1) participants viewed 120 pictures of landscapes and living room interiors for 1.5 s each (ISI 1-1.5 s). After a short break, immediate recognition was tested by presenting the learned pictures together with 120 new pictures. The discrimination index d-prime was used as measure of learning performance. In the number learning task (adapted from [16]) 16 three digit numbers were presented three times each for 2 s (500 ms ISI). A minute after presentation, participants were asked to freely recall the original numbers and free recall was used as a control measure of non-specific effects on learning after sleep. Afterwards, recognition of the numbers intermixed with 16 new three digit numbers was assessed, and d-prime was calculated as additional dependent measure.

Statistical Analyses

Data from three participants were excluded due to poor sleep (two from Experiment 1 and one from Experiment 2). One additional participant was excluded from the sleep analyses in Experiment 2 due to missing data during one night and an additional participant was excluded from Experiment 1 as he had been allowed to sleep an hour longer in the fenobam condition by accident. In Experiment 1, the analyses used repeated measures ANOVAs with the factor Time point (reflecting performance at Learning and Retrieval phases), and Treatment

(fenobam and placebo). In Experiment 2, we used factors for Interference (reflecting performance on the A-C interference word-pairs and the D-E no interference word-pairs), for Blocks (for learning block 1-3) and Treatment (fenobam and placebo). Post-hoc comparisons and other pairwise comparisons relied on dependent measures t-tests. Greenhouse-Geisser corrected degrees of freedom were used where appropriate. Cluster-based non-parametric permutation tests with correction for multiple comparisons across channels and frequency bins as implemented in FieldTrip [52] were conducted to compare EEG power spectra under fenobam and placebo (Figures 3A and 4A). EEG measures from oscillatory event detection analyses (density, amplitude, frequency) were averaged across channels and subjected to two-sided paired t-tests contrasting fenobam and placebo conditions per frequency band (Figures 3C and 4C). For PAC analyses, Rayleigh tests were used to test for significant clustering of phase angles (deviation from uniform distribution) across subjects per condition, and two-sided paired t-tests were conducted to contrast mean vector length (Figure 3F) and average absolute deviation from group average (Figure 3G) between fenobam and placebo conditions.

Results

Memory tasks

In general, there were no significant effects of the treatment on measures of memory retention (all $p > 0.14$; see Figure 1B and Tables 1 and 2). Specifically, In Experiment 1, participants correctly recalled more word-pairs during the Retrieval phase than during the Learning phase ($F_{(1,16)} = 137.52$, $p \leq 0.001$). None of the Treatment-related main or interaction effects were significant ($p \geq 0.61$). Similarly, on the finger sequence tapping task, participants tapped more correct finger sequences during the Retrieval phase ($F_{(1,16)} = 24.71$, $p \leq 0.001$), without any Treatment-related effects becoming apparent for correct sequences or the error rate ($p \geq 0.39$). Participants increased the number of correctly tapped finger sequences during the for the control sequence learned after retrieval in Experiment 1 across the twelve blocks ($F(3,48) = 21.10$, $p \leq 0.001$). But there were no further effects evident for the number of correct sequences or the error rate ($p \geq 0.27$).

In Experiment 2, participants recalled fewer word-pairs during the Retrieval phase than during the Learning phase ($F_{(1,18)} = 126.90$, $p \leq 0.001$). Note, the generally worse recall in Experiment 2 than Experiment 1 is due to the procedure of Experiment 2 including, before the Retrieval phase, the New Learning of word-pairs half of which interfered (i.e., shared the same cue word) with the word-pairs learned during the Learning phase before sleep. Accordingly, there was also a main effect of interference ($F_{(1,18)} = 7.28$, $p = 0.015$) that was due to participants performing worse on retrieval of the interference word-pairs compared to the no-interference word-pairs during the Retrieval phase (time point x interference interaction: $F_{(1,18)} = 6.92$, $p = 0.017$). None of the Treatment-related effects was significant ($p \geq 0.14$).

There were also no significant effects of Treatment on learning performance during the New Learning phase after sleep, in general (see Figure 1C and Tables 1 and 2 for descriptive statistics). In detail, during the New Learning of word pairs in Experiment 2 participants

increased their performance across the three blocks ($F_{(2,36)} = 355.56$, $p \leq 0.001$), but none of the other effects was significant ($p \geq 0.11$).

Sleep stages

Overall, fenobam severely impacted gross sleep architecture compared to placebo, when analysing time spent in each sleep stage relative to total sleep time (TST) during each night (Figure 2A and Table 3). The statistical analyses yielded the same results for absolute time in minutes per sleep stage, and consistent Treatment effects in both experiments. In Experiment 1, fenobam increased time spent in wakefulness and NonREM1 (wakefulness: $t_{(16)} = -1.78$, $p = 0.095$, NonREM1: $t_{(16)} = -3.23$, $p = 0.005$) in favour of NonREM 2, NonREM3 and REM sleep (NonREM2: $t_{(16)} = 2.33$, $p = 0.033$, NonREM3: $t_{(16)} = 3.11$, $p = 0.007$, $p = 0.016$, REM: $t_{(16)} = 2.94$, $p = 0.010$). NonREM4 and TST seemed unaffected by treatment ($p \geq 0.26$). In Experiment 2, similarly, under fenobam participants spent more time in wakefulness and NonREM1 (wakefulness: $t_{(17)} = -3.24$, $p = 0.005$, NonREM1: $t_{(17)} = -4.29$, $p \leq 0.001$) and less time in NonREM2 and NonREM3 (NonREM2: $t_{(17)} = 3.57$, $p \leq 0.001$, NonREM3: $t_{(17)} = 2.04$, $p = 0.057$). Also, in Experiment 2 there was a trend towards participants sleeping less under fenobam than placebo (TST: $t_{(17)} =$, $p = 0.089$), but NonREM4 again stayed unaffected ($p = 0.41$).

Pooling sleep data from both experiments, to take advantage of the increased sample size, provided further evidence that fenobam increases wakefulness and NREM1 (wakefulness: $t_{(34)} = -2.94$, $p = 0.006$, NonREM1: $t_{(34)} = -5.20$, $p \leq 0.001$), while decreasing NREM2, NonREM3 and REM sleep (NonREM2: $t_{(34)} = 4.04$, $p \leq 0.001$, NonREM3: $t_{(34)} = 3.50$, $p \leq 0.001$, REM: $t_{(34)} = 3.10$, $p = 0.004$). It also provided more evidence for a general decrease in total sleep time under fenobam (TST: $t_{(34)} = 2.16$, $p = 0.038$). Even with this increased power to detect differences time spent in NonREM4 was not affected significantly ($p = 0.59$).

Power spectra, event detection, and phase-amplitude coupling (PAC) analyses

During NonREM stages 2-4 sleep fenobam (relative to placebo) suppressed spindle band (12-15 Hz) power but increased delta band (2-4 Hz) power in all channels ($p_{corr} < 0.05$; Figure 3A and Supplementary Figure S1). During REM sleep, theta band (4-8 Hz) and beta

band (16-22 Hz) power were suppressed and alpha band (10-12 Hz) power was increased in all channels after fenobam ($p_{corr} < 0.05$; Figure 4A and Supplementary Figure S2). Thanks to the applied normalization and 1/f-correction procedure, these findings cannot be attributed to changes in broadband power but have to originate from fenobam induced modulations of distinct oscillations in specific frequency bands.

This notion was further corroborated by the oscillatory event detection analyses for these bands. During NonREM sleep (Figure 3B-C), fenobam indeed increased the amplitude of delta waves ($t_{23} = -4.21$, $p = 0.0003$) while decreasing sleep spindle amplitude ($t_{23} = 5.53$, $p < 0.0001$). In addition, fenobam also reduced the density of distinct SOs ($t_{23} = 2.26$, $p = 0.03$) and spindle events ($t_{23} = 2.50$, $p = 0.02$) and increased spindle frequency ($t_{23} = -3.93$, $p = 0.0008$). During REM sleep (Figure 4 B-C), fenobam reduced the amplitude of theta ($t_{23} = 3.78$, $p = 0.001$), alpha ($t_{23} = 6.27$, $p < 0.0001$) and beta ($t_{23} = 2.94$, $p = 0.007$) waves as well as the density of beta waves ($t_{23} = 3.99$, $p = 0.0006$). Thus, only for the REM alpha oscillations there was an apparent mismatch between an increase in band power but a decrease in event amplitude, so that no unambiguous direction of modulation could be deduced for alpha oscillations.

We also tested whether the SO-spindle PAC was affected, which is thought to be most crucial for effective memory processing. Rayleigh tests confirmed significant clustering of spindle peaks at comparable phase-angles under fenobam (311°, $z = 15.08$, $p < 0.00019$) and placebo (314°, $z = 9.62$, $p < 0.0000001$). However, a larger mean vector length ($t_{23} = 3.89$, $p < 0.001$) and a smaller absolute deviation of individual preferred phase from the group angular mean was observed under fenobam than placebo ($t_{23} = 2.63$, $p = 0.015$), indicating that fenobam actually increased the precision and consistency of SO-spindle PAC both within and across subjects.

Control measures

In Experiment 1, participants subjectively felt more sleepy in the morning after sleep under fenobam than under placebo according to the Stanford Sleepiness Scale (wakefulness: $t_{(16)} =$

-2.34, $p = 0.033$), which was not the case in Experiment 2, at this time point, or at any other time points in both experiments ($p \geq 0.36$). Objective measures of reaction speed (i.e., one divided by the individual average reaction time) on the psychomotor vigilance task did not show any treatment effects ($p \geq 0.17$). The multidimensional mood questionnaire showed increased tiredness and reduced positive affect in the fenobam conditions directly after awakening in Experiment 1 (tiredness: $t_{(16)} = 2.09$, $p \leq 0.053$, positive affect: $t_{(16)} = 4.908$, $p \leq 0.001$). In Experiment 2, we found a baseline difference of reduced positive affect and feeling calm in the fenobam condition (positive affect: $t_{(17)} = 2.70$, $p \leq 0.015$, feeling calm: $t_{(17)} = 2.25$, $p \leq 0.037$), a trend towards lower positive affect in the fenobam condition directly after awakening (positive affect: $t_{(17)} = 1{,}77$, $p = 0.094$) and a reduced feeling of calmness after the Retrieval phase (feeling calm: $t_{(17)} = 2.11$, $p = 0.049$). All in all, any effects found in the control tasks were rather small und unsystematic, except for increased sleepiness at awakening in Experiment 1.

Concerning general retrieval performance measured by the word generation task during the Retrieval phase, there were no significant effects of treatment in Experiment 1 or Experiment 2 ($p \geq 0.18$). When recognising numbers, participants made less false alarms in the placebo condition than in the fenobam condition in the New Learning phase of Experiment 1 ($t_{(16)} = -2.14$, $p = 0.048$), which was not replicated in the New Learning phase 2 of Experiment 2 ($t_{(18)} \leq 0.01$, $p \geq 0.99$). No other treatment effects were evident for the number learning task regarding recognition or free recall in Experiment 1 or Experiment 2 ($p \geq 0.19$). There were also no significant treatment effects on recognising pictures in the New Learning phase of Experiment 1 ($p \geq 0.64$).

There was no evidence of increased adverse side effects under fenobam in Experiment 1 or Experiment 2 at any point in time ($p \geq 0.17$). However, in Experiment 2, there was a trend towards participants being able to guess whether they had received fenobam or placebo (Exact McNemar, $p = 0.092$) and this observation received further support when combining data from both experiments (Exact McNemar, $p = 0.023$).

Discussion

Sleep benefits the formation of long-term memory by facilitating consolidation and new learning [53]. Sleep-dependent declarative memory consolidation has been shown to essentially rely on neuronal replay that is thought to lead to systems consolidation [2,6], whereas new learning after sleep has been proposed to benefit from synaptic renormalisation and presumed forgetting of irrelevant memories [3,28,54]. In the present study, we continue our efforts to identify the putative glutamatergic processes that are essential for these plastic processes at the synaptic level [16]. Specifically, we hypothesised that blockade of mGluR5 during sleep by fenobam would impair replay-dependent synaptic consolidation processes. Moreover, given that these receptors might also be involved in synaptic depotentiation in hippocampal networks [55] we explored whether the blocker would also impair new learning and encoding into hippocampal networks. To this end, participants learned word-pairs before sleep and retrieved them thereafter to measure retention in Experiment 1 and learned new word-pairs after sleep in Experiment 2, while receiving the selective mGluR5 blocker fenobam or placebo shortly before sleep. As expected, participants increased the number of recalled word-pairs across sleep in Experiment 1 as well as across learning blocks in Experiment 2. However, these processes were not impacted by fenobam. Interestingly, blocking mGluR5 had a strong effect on gross sleep architecture including sleep EEG oscillations closely linked to sleep-dependent memory processing. The mGluR5 blocker increased wakefulness and NonREM1 in favour of NonREM2, NonREM3 and REM sleep. More detailed analyses revealed that fenobam suppressed 12-15 Hz sleep spindles and augmented 2-4 Hz, i.e., un-physiologically high, delta waves during NonREM, while suppressing mainly theta and beta waves during REM sleep. Notably, spindles became even more consistently coupled to slow oscillation phase, potentially compensating for their overall reduced number.

Glutamate is the most abundant excitatory neurotransmitter and glutamatergic LTP is the most intensively studied form of synaptic plasticity that leads to learning in neuronal systems [18]. To our surprise, we did not find evidence that blocking AMPA or NMDA receptors impairs sleep-dependent declarative memory consolidation [16]. One possible explanation for

this may be that the functional role of the NMDA receptor in mediating enhanced excitability shifts to other glutamatergic receptors during sleep. The mGluR5 is an ideal candidate for such a shift, as it is functionally coupled to the NMDA receptor [56]. In line with this argument, mGluR5 receptor availability increases during sleep deprivation in healthy humans [24], putatively acting as a tag for sleep-dependent consolidation. However, since we could not provide evidence for impaired sleep-dependent declarative memory consolidation by blocking mGluR5 such a shift seems unlikely to have acted here.

Not only synaptic potentiation but also depotentiation has been shown to rely on activation of mGuR5 [22,55,56]. Similar to our findings regarding ketamine it is plausible that by blocking potentiation and depotentiation at the same time we received a null effect on the retention data. To answer this question we conducted Experiment 2, in which participants learned new word-pairs after sleep. Here, we also found no effect of mGluR5 blockade so this line of argument is refuted. Importantly, Experiment 2 also allowed us to conceptually replicate our null effect on retention in Experiment 1 with an interference paradigm, which has been shown to be sensitive to the memory enhancing effect of sleep [57].

Power is important when judging null effects. In this regard, the chosen sample size (n = 20 per experiment in a within-subject design) was a significant improvement compared to similar approaches of pharmacologically investigating sleep-dependent glutamatergic memory processes in humans (e.g., [16,19]) and constitutes a major experimental effort. We chose two procedures to measure declarative memory processing across sleep that have previously been shown to be highly susceptible to manipulations of sleep, yielding large effect sizes [35-37,57]. After excluding participants due to sleep problems we achieved reasonable power of 0.62 and 0.67 for Experiment 1 and Experiment 2 respectively (assuming a medium effect size of $d = 0.5$ and a two-sided type one error rate of $\alpha = 0.05$, calculated in G*Power 3.1.7 [58]). When assessing both experiments together this yields the combined type two error rate of 12.54% for falsely retaining the null hypothesis that there is no medium to large effect of mGluR 5 blockade on memory processes in our experiments. To sum up, although smaller effects

cannot reasonably be ruled out, we are confident that mGluR 5 mediated processes do not play a major role for sleep-dependent declarative memory processes.

Importantly, our finding that fenobam severely disrupted both REM and NonREM sleep and changed sleep-specific oscillatory activity, indicates that sufficient levels of fenobam arrived at the brain. Although effects of manipulating mGluR5 on sleep have been described in animal studies, they do not completely correspond to our findings in humans. In fact, some of findings in rodents suggest the exact opposite relationship between mGluR5 activity and sleep (oscillatory activity) than what we observed in humans. Ahnaou and colleagues found that mGluR5 knock-out mice had a preserved circadian sleep-wake architecture, reduced REM sleep and reduced 1-4 Hz delta power during NonREM sleep, as well as reduced sleep drive and SWA (0.5-4 Hz) after sleep deprivation [59]. In rats, mGluR5 negative allosteric modulators also suppressed REM sleep, but increased and consolidated NonREM sleep [60,61], whereas mGluR5 positive allosteric modulators increased waking, and decreased both REM and NonREM sleep [60]. Others reported mGluR5 to be wake promoting in general [62].

In line with a sleep regulatory role of mGluR 5, a recent positron emission tomography (PET) study in humans, using a highly selective, non-competitive radio-ligand, showed increased mGluR5 availability after sleep loss [63]. This increase was correlated to behavioural and EEG biomarkers of elevated sleep need, with mGluR5 availability being positively related to NonREM slow wave activity (0.5-4.5 Hz), both at baseline and after sleep deprivation. In this sense, mGluR5 mediated activity seems to support homeostatic increases in SWA in humans, which is reconcilable with the reduced time spent in SWS after blocking these receptors with fenobam in our study. Note, that we also found the density of SOs (0.5-1.25 Hz) to be reduced, but the amplitude of delta waves (1-4 Hzto be increased under fenobam. While corroborating the different nature of these two oscillations [64], it complicates a direct comparison with studies for which SO and delta were lumped together as slow wave activity (SWA; 0.5-4.5 Hz). We speculate that the spectrally circumscribed increase in delta power, resulted from an increase in thalamically, as opposed to cortically, generated slow waves [64]. While it is very

plausible that metabotropic glutamate receptors in the thalamus and cortico-thalamic feedback mechanisms are involved in the observed effects of fenobam on spindle decreases and delta increases, the direction of the effect is again opposed to what would have been predicted from previous animal work. Corticothalamic activation of metabotropic glutamate receptors in the thalamus of guinea pigs induced a slow depolarization via reduced potassium currents, shifting the burst firing mode of thalamic relay cells, characteristic for SWS, to the single spiking mode dominating wakefulness [65], and corticothalamic feedback has specifically been shown to be capable of flipping thalamic activity from spindle bursts to ~3 Hz delta bursts (even though metabotropic glutamate receptors were not considered in those studies) [66,67]. It therefore remains unclear why in humans blocking of mGluR5 (instead of its activation) caused exactly such a shift from spindle to delta waves. The present findings thus corroborate the idea that mGluR5 are significantly involved in the regulation of sleep and that they exert their influence on sleep specific oscillatory activity via cortico-thalamic-feedback loops. However, the direction of the effects observed in humans and rodents is not easily reconciled, and specific studies aimed at disentangling the precise mechanisms at play are needed.

Irrespective of the precise pharmacological and neurophysiological underpinnings of the observed spindle suppression, the question remains why a significant decrease in spindle amplitude and density did not affect memory consolidation. Recent work has demonstrated that memory consolidation relies in particular on spindles occurring during SO up-states, reflected by SO-spindle-PAC [44,68-70]. Notably, SO-spindle-PAC was not only preserved under fenobam, but became even more pronounced, both within and across subjects. Unlike their overall reduction in power and density, the increased synchronization of spindles to SO up-states is well in line with a fenobam-induced antagonism of mGluR5 receptors in the thalamus, which is supposed to facilitate the SWS-like burst firing mode of thalamocortical relay cells [65].

In conclusion, our experiments constitute a yet another failure to find essential contributions of glutamatergic processes to sleep-dependent memory formation that adds to

our finding that AMPA and NMDA receptor blockade does not impair sleep-dependent declarative memory consolidation [16]. This remains an especially unexpected finding considering the importance of glutamatergic contributions to neurotransmission, neuroplasticity and learning, which makes it important to emphasize that for example blockade of NMDA-receptor subunits (e.g., NR2B) or simultaneous blockade of NMDA and mGlu 5 receptors could be a more promising approach. However, this can only be done in animal models. While it may seem that our work is marginalising glutamate, quite to the contrary, it is possible that glutamatergic contributions to sleep-dependent memory processes are so important for an organism's survival that there exist several redundant signalling pathways. In addition, our findings of sleep disruption, reduced sleep-spindle activity and increases in un-physiological high delta underline the importance of mGluR5 for sleep regulation. Considering current efforts to develop mGluR5 related drugs [71], it will be highly important to characterise sleep disturbance (or improvement) related to these drugs as part of their neuropsychopharmacological profile.

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Figure Legends

Figure 1 A) Time course of Experiment 1 and Experiment 2 (see Methods section for details). B) Correctly recalled word-pairs (A-B) during the Retrieval phase relative to the Learning phase in Experiment 1 (left panel). Correctly tapped finger sequences of the three blocks during the Retrieval phase relative to the last three blocks during the Learning phase in Experiment 1 (middle panel). Correctly recalled word-pairs during the Retrieval phase relative to the Learning phase in Experiment 2 for interference word-pairs (A-B), no interference word-pairs (A-B) and total (A-B) word-pairs (right panel). C) Mean (SEM) correctly recalled interfering (A-C) and non-interfering (E-D) word-pairs during the New Learning 1 phase in Experiment 2 (left and middle lower panel). Red and blue Box and whisker plots (thick line: median, hinge: 25% and 75% quartiles, whiskers: smallest/largest values no further than 1.5 * inter quartile range from the hinge) indicate fenobam and placebo, respectively.

Figure 2 Mean (SEM) time spent in the different sleep stages (W – wake, N1-4 – NonREM1-4, SWS – slow wave sleep, REM – rapid eye movement sleep) relative to total sleep time for Experiment 1 (left panel), Experiment 2 (middle panel) and both experiments combined (right panel). Red and blue Box and whisker plots (thick line: median, hinge: 25% and 75% quartiles, whiskers: smallest/largest values no further than 1.5 * inter quartile range from the hinge) indicate fenobam and placebo respectively. (* $p \leq 0.05$, ** $p \leq 0.01$)

Figure 3 (A) Normalized 1/f-corrected power spectra during NonREM sleep (stages 2-4) reveal spindle (12-15 Hz) and theta (4-8 Hz) power decrease and delta power (2-4 Hz) increase after fenobam (red) compared to placebo (blue) intake (all 9 channels are shown in Supplementary Figure S1). Inserts provide zoom into outlined (dashed lines) areas to highlight delta power increases. Horizontal black bars and shaded area between lines indicate significant differences between fenobam and placebo (corrected for multiple comparisons across all electrodes and frequency bins using cluster-based permutation statistics). (B) Grand average time-locked waveforms for slow oscillations, delta waves, spindles resulting from event detection. (C) As revealed by event detection, under fenobam compared to placebo, slow oscillation and spindle

density were reduced, spindle amplitude was decreased, delta wave amplitude increased, and spindles became slightly faster. Asterisks indicate significant differences at $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***). (D) Angular histograms for a single representative participant illustrate slow oscillation to spindle phase-amplitude coupling (SO-spindle PAC) under fenobam (right) and placebo (left). (E) Preferred SO-spindle PAC phase (indicated by small circles on unitary circle) and their circular phase average across subjects (indicated by vector) reveal phase clustering across participants indicating spindle maxima during the slow oscillation down-to-up transition. Preferred SO-spindle PAC phase was comparable for fenobam (311°) and placebo (314°), but under fenobam SO-spindle PAC it was more clustered, both (F) within subjects (indicated by a larger mean vector length, $p = 0.0007$) and (G) across subjects (indicated by a smaller deviation of the subjects' individual phase from the group mean, $p = 0.015$).

Figure 4 (A) Normalized 1/f-corrected power spectra during REM sleep reveal theta (4-8 Hz) and beta (16-22 Hz) power decreases and alpha power (10-12 Hz) increase (but no significant delta power increase) after fenobam (red) compared to placebo (blue) intake (all 9 channels are shown in Supplementary Figure S2). Horizontal black bars and shaded area between lines indicate significant differences between fenobam and placebo (corrected for multiple comparisons across all electrodes and frequency bins using cluster-based permutation statistics). (B) Grand average time-locked waveforms for delta, theta, and beta waves resulting from event detection. (C) As revealed by event detection, under fenobam compared to placebo, beta wave density was reduced, and theta, alpha, and beta wave amplitudes were decreased. Asterisks indicate significant differences at $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***).

Tables

Table 1: Mean (SEM) for the memory tasks in Experiment 1.

	Fenobam		Placebo	
Word-pairs				
Learning	47.29	(5.17)	45.94	(5.62)
Retrieval	68.18	(4.94)	68.00	(5.12)
Difference	20.88	(1.52)	22.06	(2.64)
Percent	153.63	(6.80)	164.13	(13.17)
Finger sequence A				
Correct sequences				
Learning	20.63	(0.95)	20.69	(1.29)
Retrieval	23.33	(1.39)	23.82	(1.69)
Difference	2.71	(0.64)	3.14	(0.63)
Percent	112.51	(2.64)	114.83	(3.10)
Error rate				
Learning	0.08	(0.01)	0.09	(0.02)
Retrieval	0.06	(0.01)	0.08	(0.02)
Difference	-0.02	(0.01)	-0.01	(0.02)
Finger sequence B				
Correct sequences				
New learning 1-3	15.29	(1.20)	16.14	(1.38)
New learning 4-6	18.29	(1.26)	19.20	(1.29)
New learning 7-9	18.86	(1.11)	19.56	(1.15)
New learning 10-12	19.64	(1.37)	19.35	(1.21)
Error rate				
New learning 1-3	0.11	(0.01)	0.13	(0.03)
New learning 4-6	0.11	(0.02)	0.09	(0.02)
New learning 7-9	0.10	(0.01)	0.08	(0.01)
New learning 10-12	0.10	(0.03)	0.09	(0.02)
Numbers				
Recognition				
D-prime	1.45	(0.20)	1.71	(0.19)
Hits	0.72	(0.04)	0.72	(0.04)
False alarms	0.23	(0.04)	0.15	(0.03)
Free recall				
Absolute	10.05	(0.72)	10.41	(0.79)
Correct	6.65	(0.95)	7.53	(0.86)
Pictures				
D-prime	1.89	(0.18)	1.97	(0.19)
Hits	0.80	(0.04)	0.80	(0.04)
False alarms	0.20	(0.03)	0.19	(0.03)
Word generation				
Letter	17.17	(1.08)	16.53	(0.72)
Category	21.82	(1.22)	20.00	(0.84)
Total	39.00	(1.77)	36.53	(1.22)

Table 2: Mean (SEM) for the memory tasks in Experiment 2.

	Fenobam		Placebo	
Word-pairs				
Interference (A-B)				
Learning	29.31	(0.72)	29.63	(0.78)
Retrieval	23.32	(0.72)	22.68	(1.11)
Difference	-6.00	(0.80)	-6.95	(0.74)
Percent	79.17	(2.88)	76.24	(2.70)
Blocks	2.10	(0.19)	2.10	(0.15)
No Interference (A-B)				
Learning	30.47	(0.81)	31.73	(0.94)
Retrieval	26.16	(0.94)	26.68	(0.94)
Difference	-4.32	(0.75)	-5.05	(0.66)
Percent	86.04	(2.45)	84.20	(2.04)
Blocks	2.21	(0.20)	2.21	(0.18)
Total				
Learning	59.79	(0.84)	61.37	(1.14)
Retrieval	49.47	(1.44)	49.37	(1.55)
Difference	-10.32	(1.16)	-12.00	(1.09)
Percent	82.68	(2.02)	80.36	(1.85)
Interference (A-C)				
New learning 1	17.21	(1.44)	17.05	(1.48)
New learning 2	26.79	(1.46)	27.95	(1.33)
New learning 3	32.42	(1.40)	33.47	(0.97)
No Interference (D-E)				
New learning 1	15.32	(1.81)	16.32	(1.53)
New learning 2	26.63	(1.89)	28.16	(1.87)
New learning 3	32.52	(1.77)	33.63	(1.24)
Total				
New learning 1	32.52	(3.09)	33.37	(2.70)
New learning 2	53.42	(3.12)	56.10	(2.87)
New learning 3	64.94	(3.00)	67.11	(1.96)
Numbers				
Recognition				
D-prime	0.92	(0.13)	1.05	(0.16)
Hits	0.62	(0.03)	0.67	(0.03)
False alarms	0.30	(0.03)	0.30	(0.03)
Free recall				
Absolute	8.11	(0.77)	7.68	(0.65)
Correct	4.47	(0.56)	4.21	(0.49)
Word generation				
Letter	15.26	(1.09)	15.94	(1.00)
Category	19.42	(1.12)	20.47	(0.84)
Total	34.68	(1.99)	36.42	(1.43)

Table 3: Mean (SEM) for the sleep stages and the control measures. Note: Tiredness (low = tired), Positives affect (high = positive), Feeling calm (high = calm).

	Experiment 1				Experiment 2			
	Fenobam		Placebo		Fenobam		Placebo	
Sleep stages								
Minutes								
Wake	35.53	(7.32)	20.50	(3.86)	19.00	(4.22)	7.94	(1.81)
NonREM1	77.03	(15.72)	30.50	(2.66)	75.08	(10.65)	40.33	(4.69)
NonREM2	220.97	(13.66)	258.47	(6.17)	213.75	(11.70)	257.58	(6.55)
NonREM3	33.91	(4.64)	43.47	(5.39)	45.19	(3.98)	54.08	(2.89)
NonREM4	9.00	(3.13)	10.15	(3.07)	26.61	(5.34)	24.22	(5.19)
SWS	42.91	(6.78)	53.62	(7.55)	71.81	(7.23)	78.31	(5.31)
REM	78.44	(8.78)	101.62	(5.24)	73.14	(6.34)	85.00	(6.34)
TST	462.44	(4.82)	469.06	(3.21)	456.81	(9.54)	471.67	(3.65)
Percent of TST								
Wake	7.80	(1.65)	4.41	(0.84)	4.15	(0.91)	1.67	(0.38)
NonREM1	16.56	(3.28)	6.51	(0.56)	16.59	(2.33)	8.51	(0.91)
NonREM2	47.75	(2.87)	55.06	(1.18)	47.03	(2.55)	54.65	(1.42)
NonREM3	7.36	(1.00)	9.25	(1.14)	9.79	(0.80)	11.46	(0.61)
NonREM4	1.99	(0.71)	2.16	(0.66)	5.69	(1.14)	5.09	(1.11)
SWS	9.37	(1.50)	11.42	(1.59)	15.49	(1.47)	16.56	(1.11)
REM	16.90	(1.84)	21.66	(1.08)	15.83	(1.84)	18.08	(1.36)
Reaction speed (1/ms)								
Learning phase	3.93	(0.07)	3.84	(0.09)	3.77	(0.10)	3.76	(0.10)
Retrieval phase	3.96	(0.09)	3.97	(0.10)	3.77	(0.11)	3.77	(0.10)
Sleepiness								
Learning phase	3.94	(0.30)	3.82	(0.23)	2.89	(0.21)	3.16	(0.21)
Awakening	4.12	(0.30)	3.35	(0.30)	3.21	(0.27)	2.95	(0.25)
Retrieval phase	2.24	(0.20)	2.47	(0.27)	2.32	(0.32)	2.21	(0.27)
Adverse effects								
Learning phase	2.29	(0.44)	2.47	(0.30)	1.73	(0.35)	2.16	(0.49)
Awakening	3.35	(0.66)	2.53	(0.40)	1.58	(0.36)	1.63	(0.39)
Retrieval phase	1.88	(0.44)	1.18	(0.38)	1.53	(0.50)	0.95	(0.32)
Tiredness								
Learning phase	2.62	0.20	2.66	0.18	3.22	0.17	3.36	0.12
Awakening	2.75	0.20	3.04	0.21	3.14	0.18	3.37	0.17
Retrieval phase	3.56	0.20	3.59	0.25	3.65	0.25	3.78	0.23
Positive affect								
Learning phase	3.63	0.13	3.42	0.20	4.13	0.08	4.48	0.08
Awakening	3.06	0.15	3.69	0.12	3.72	0.23	4.25	0.16
Retrieval phase	3.66	0.16	3.78	0.15	4.18	0.23	4.51	0.08
Feeling calm								
Learning phase	3.83	0.16	3.62	0.21	3.88	0.18	4.18	0.16
Awakening	3.72	0.18	3.99	0.16	3.51	0.22	3.93	0.20
Retrieval phase	4.06	0.13	4.03	0.17	3.76	0.20	4.22	0.13
Medication guess								
Fenobam	11		6		13		6	
Placebo	5		12		6		13	

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Figure 1

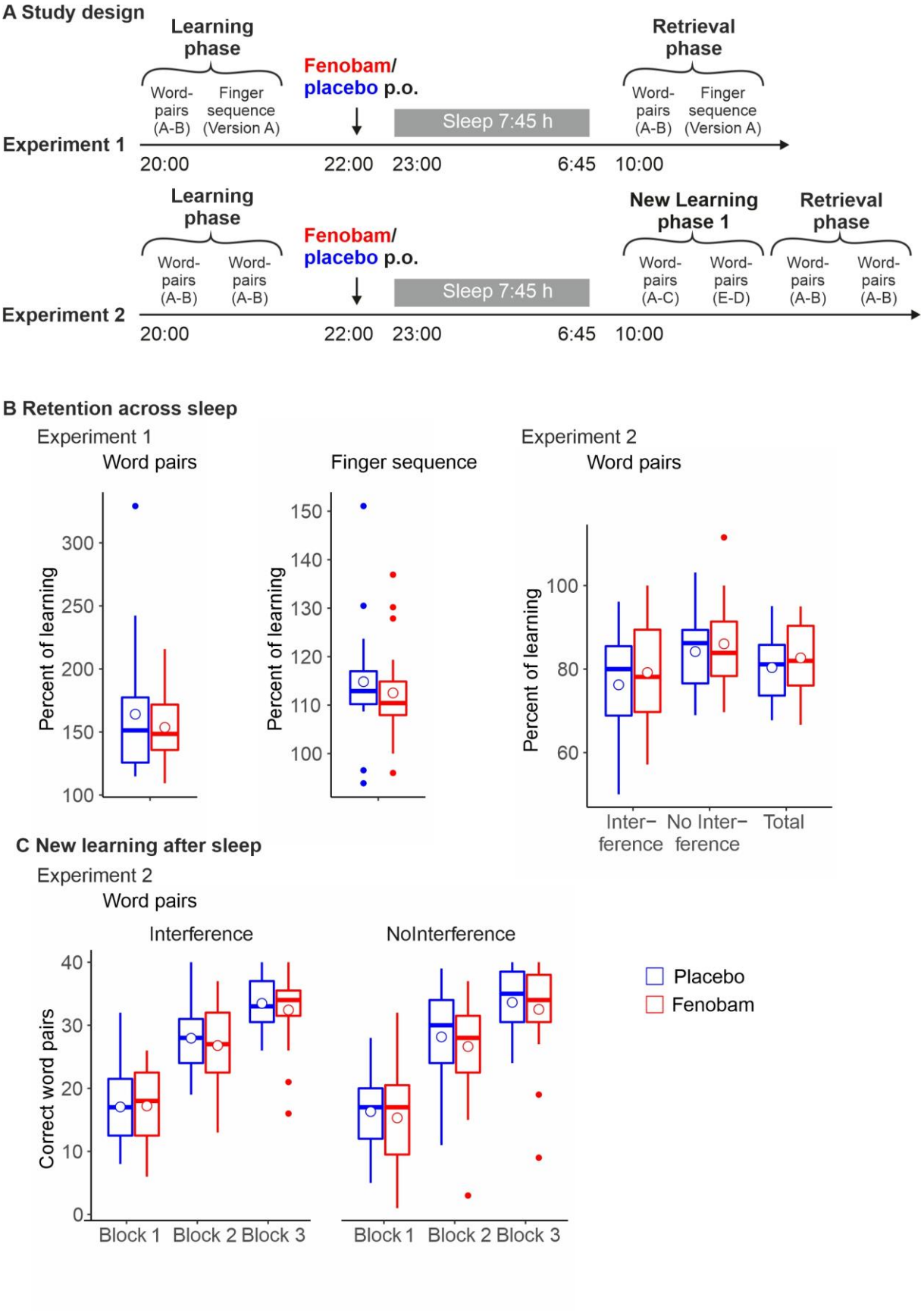
A Study design
Learning phase
Word-pairs (A-B)
Finger sequence (Version A)
Fenobam/ placebo p.o.
Sleep 7:45 h
Retrieval phase
Word-pairs (A-B)
Finger sequence (Version A)
Experiment 1
20:00
22:00
23:00
6:45
10:00
Learning phase
Word-pairs (A-B)
Word-pairs (A-B)
Fenobam/ placebo p.o.
Sleep 7:45 h
New Learning phase 1
Word-pairs (A-C)
Word-pairs (E-D)
Retrieval phase
Word-pairs (A-B)
Word-pairs (A-B)
Experiment 2
20:00
22:00
23:00
6:45
10:00
B Retention across sleep
Experiment 1
Word pairs
Finger sequence
Experiment 2
Word pairs
Percent of learning
Inter-ference
No Inter-ference
Total
C New learning after sleep
Experiment 2
Word pairs
Interference
NoInterference
Correct word pairs
Block 1
Block 2
Block 3
Placebo
Fenobam

Figure 2

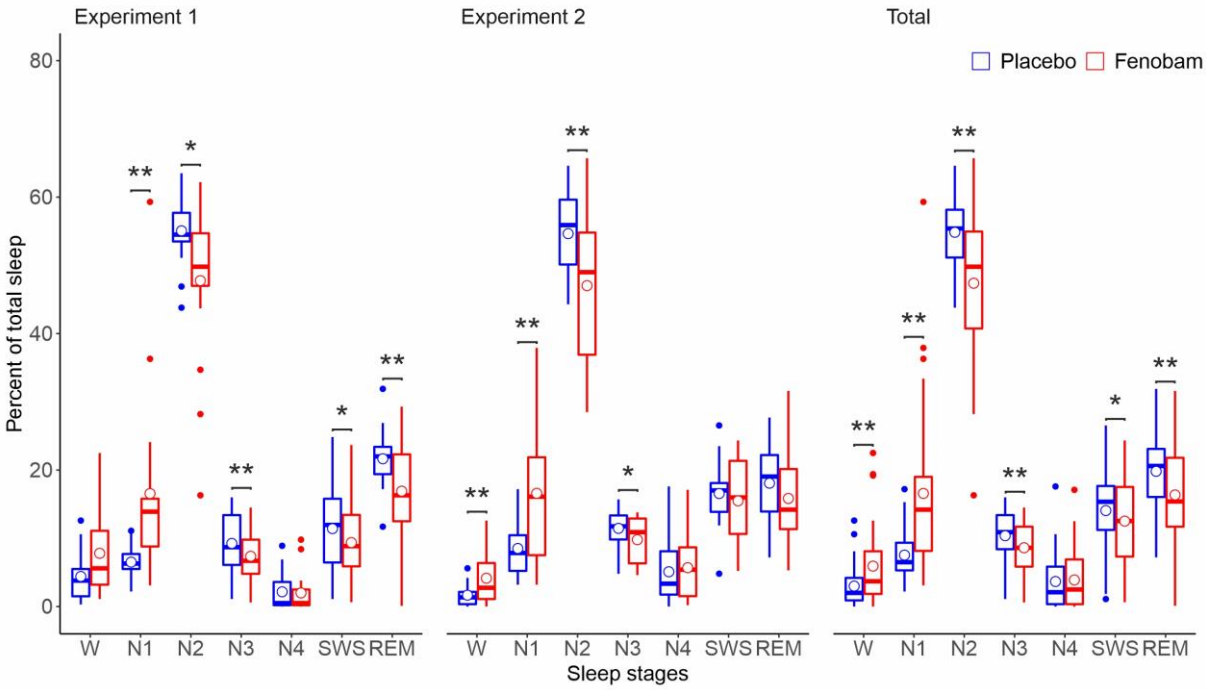

Figure 3

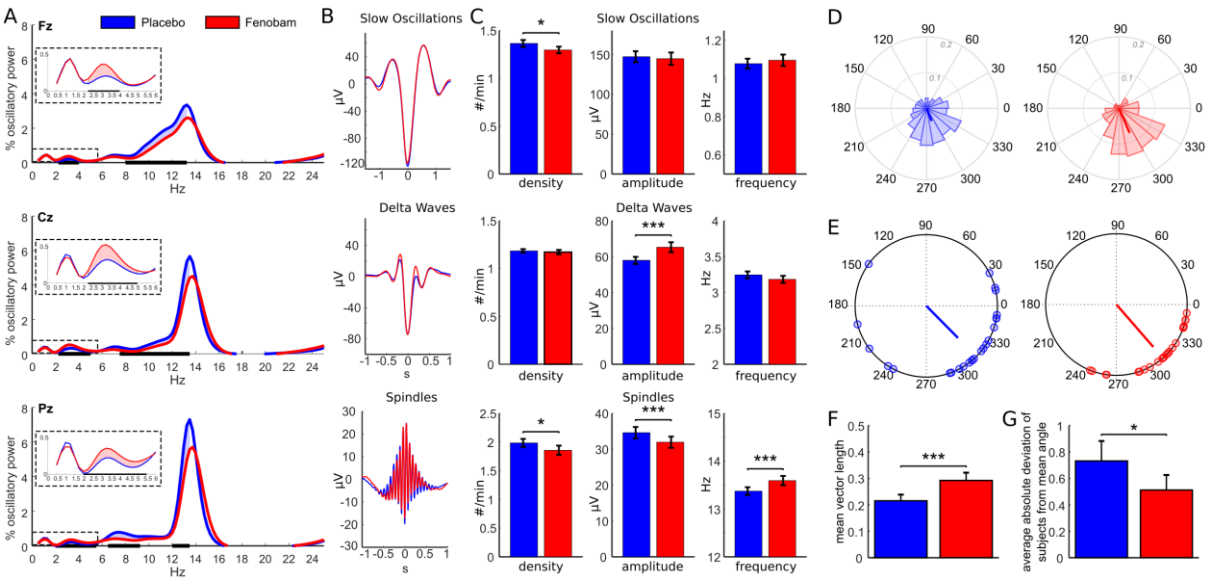

Figure 4

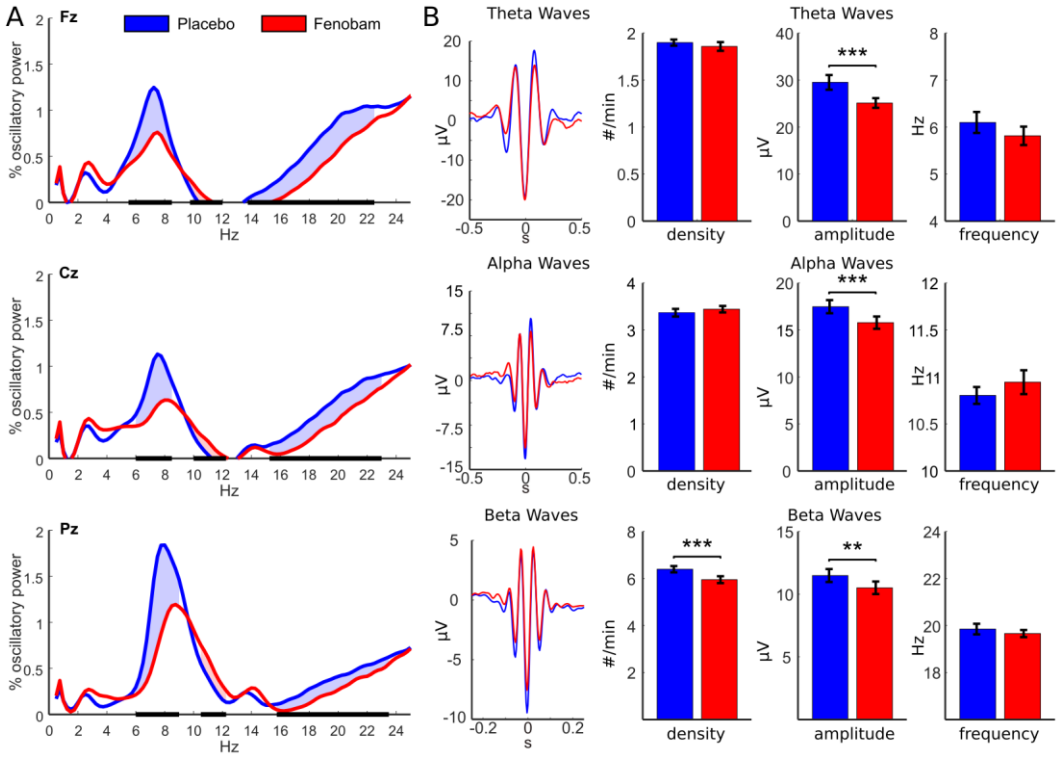
A
Fz
Placebo
Fenobam
% oscillatory power
Hz
Cz
Pz
B
Theta Waves
Alpha Waves
Beta Waves
μV
s
#/min
density
amplitude
frequency
Hz
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Figure S1- Frequency domain NonREM sleep

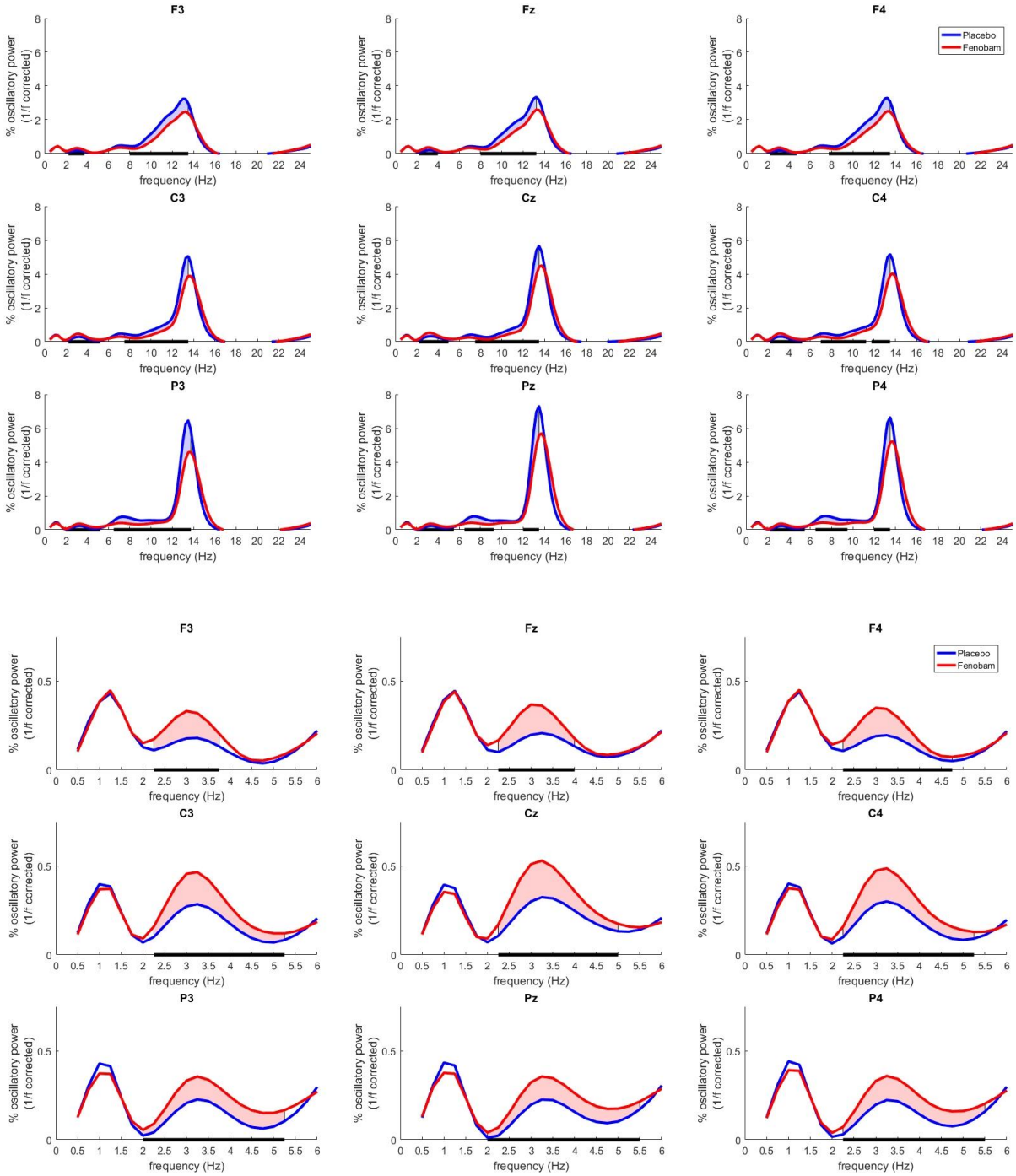
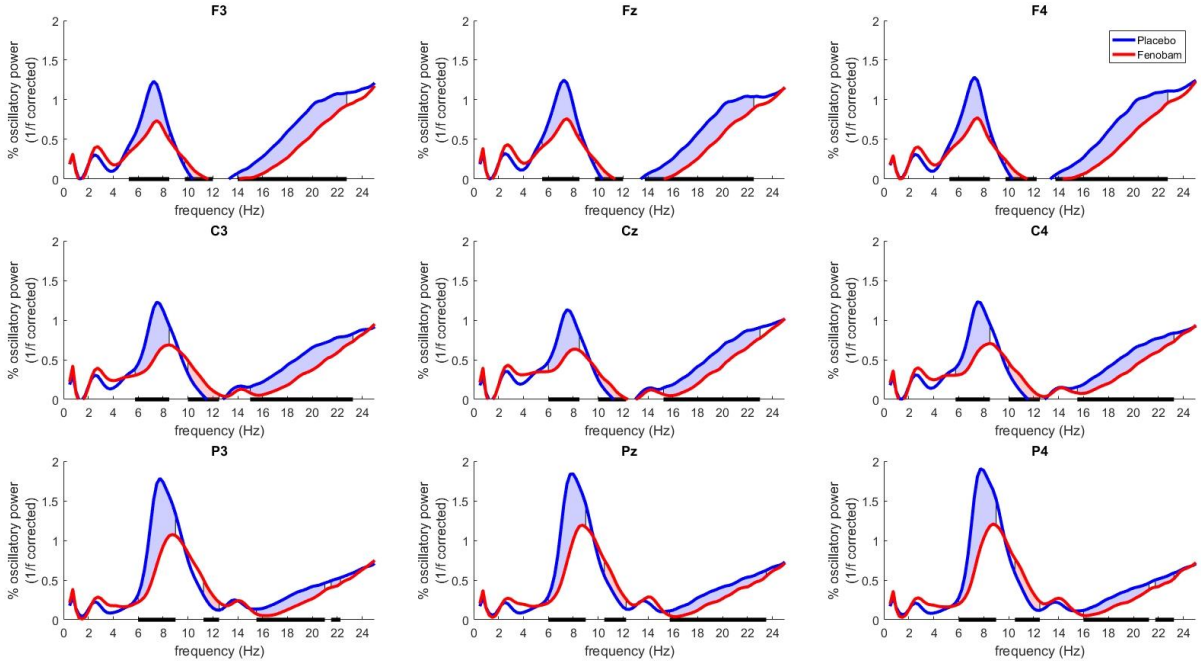
Figure S2 - Frequency domain REM sleep