Cortical oscillations to measure anti-epileptic drug activity in clinical trials

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Research Article

Keywords: TMS-induced oscillations, antiepileptic drugs, epilepsy, electro-diagnostic markers, pharmacodynamic

Posted Date: January 6th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-121959/v1

Cortical oscillations to measure anti-epileptic drug activity in clinical trials

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Number of words excluding subtitles and figures legend: 4302

Number of words abstract: 192

Number of pages: 20

Number of figures: 5

Number of tables: 1

Keywords: TMS-induced oscillations, antiepileptic drugs, epilepsy, electro-diagnostic markers, pharmacodynamic

Running title: Spectral fingerprints of antiepileptic drugs

Abstract

The frequency analysis of electroencephalographic (EEG) activity, either spontaneous or evoked by transcranial magnetic stimulation (TMS-EEG), is a powerful tool to investigate changes in brain activity and excitability following the administration of antiepileptic drugs (AEDs). However, a systematic evaluation of the effect of AEDs on spontaneous and TMS-induced brain oscillations has not yet been provided. We studied the effects of lamotrigine, levetiracetam, and of a novel potassium channel opener (XEN1101) on TMS-induced and spontaneous brain oscillations in a group of healthy volunteers. Levetiracetam suppressed TMS-induced theta, alpha and beta power, whereas lamotrigine increased TMS-induced alpha power. XEN1101 decreased TMS-induced delta, theta and beta power. Resting-state EEG showed a decrease of theta band power after lamotrigine intake. Levetiracetam increased theta, beta and gamma power, while XEN1101 produced an increase of delta, theta, beta and gamma power. Different AEDs induce specific patterns of power changes in spontaneous and TMS-induced brain oscillations. Spontaneous and TMS-induced cortical oscillations represent a powerful tool to characterize the effect of AEDs on in vivo brain activity. Spectral fingerprints of specific AEDs should be further investigated to provide robust and objective biomarkers of biological effect in human clinical trials.

1. Introduction

The development of a high percentage of central nervous system (CNS) active drugs fails due to safety concerns and toxicology issues in preclinical studies. The small number of molecules that proceed through the pipeline into human research can encounter further failure due to lack of efficacy in clinical trials. These challenges to CNS drug development have caused a clear reduction in new therapeutic products in this area. Robust and objective biomarkers of target engagement in the human brain are a key to increase the probability of successful development of new compounds in human trials [1]. The evaluation of pharmacodynamic properties in vivo can be achieved with positron emission tomography (PET) or drug distribution to the CNS by analysing cerebrospinal fluid (CSF) samples; however, these methods are invasive, expensive and not always available. Therefore, the development and validation of simple, fast and reliable markers is a paramount challenge.

Transcranial magnetic stimulation (TMS) is a non-invasive brain stimulation technique which, in combination with electroencephalography (EEG), enables a fast and accurate assessment of human brain excitability in health [2,3] and pathological conditions [4]. TMS-EEG output measures can be interrogated in the time [5] and time-frequency domains [6], providing different information about cortical processes [7]. The EEG responses to TMS averaged in the time domain are called TMS-evoked EEG potentials (TEPs), which are a reliable alternating sequence of positive (P) and negative (N) peaks at approximately 25 (P25), 45 (N45), 100 (N100) and 180 (P180) milliseconds after stimulation of the human primary motor cortex (M1) [5]. Time-frequency decomposition of the TMS-EEG signal reveals TMS-induced oscillations which, in contrast with the TEP, contain information not necessarily phase locked to the stimulus [8]. Their typical profile following M1 stimulation is characterized by an early increase of delta, theta, alpha and beta band power up to 200ms, followed by alpha and beta suppression [9] (often termed de-synchronization) with a final increase in beta power [10].

Changes in TEP amplitude has emerged as an in-vivo method to measure the effects of drugs acting on the human brain. Pharmacological studies investigating the inhibitory GABAergic pathways showed that the N45 and N100 peaks are associated with GABA-A [11,12] and GABA-B [11]

receptor-mediated neurotransmission, respectively. Peaks at 30, 45 and 180 ms are sensitive to the effects of antiepileptic drugs (AEDs) targeting voltage-gated sodium channels blockers (i.e. lamotrigine and carbamazepine) and type 2A synaptic vesicles (i.e. levetiracetam) [13-15]. More recently, TEPs were implemented in a commercial Phase I clinical trial to evaluate cortical excitability impact of XEN1101, a novel AED which potentiates the open state of potassium KCNQ2/3 channels [16], which suppressed peaks at 30, 45 and 180 ms after TMS pulse.

Despite growing knowledge about the effects of antiepileptic drugs on TEPs, their impact on TMS-induced oscillations has not been systematically explored. Only the effects of specific GABAergic drugs, such as alprazolam, baclofen, diazepam and zolpidem on oscillatory responses have been studied. Results showed that TMS-induced power changes may involve different GABAergic-inhibitory mechanisms [17].

To provide more evidence and support for the use of these measurements to guide drug development, we tested how three different AEDs affect TMS-induced brain oscillatory activity. In parallel, we have also explored their modulation of spontaneous brain oscillations. Data have been acquired in two previous TMS-EEG studies, where the effects of lamotrigine, levetiracetam (experiment 1) [13] and XEN1101 (experiment 2) [14] were shown on TEPs only. Results show that different AEDs induce specific changes in brain oscillatory activity measured by resting EEG and TMS-EEG. Therefore, together with TEPs, TMS-induced oscillations are of great potential value for inferring mechanisms and confirming specific target engagement.

2. Methods

2.1 Subjects

Fifteen (mean age 25.2 ± SD 4.62) and twenty (mean age 26.6 ± 5.9) healthy male volunteers were recruited for experiments 1 and 2, respectively. Subjects were all right-handed, according to the Edinburgh Handedness Inventory (laterality score ≥ 75%) [18]. Exclusion criteria included intake of CNS active medications, recent use of any kind of drugs (including nicotine and alcohol), neurological and psychiatric disorders, and contraindications to TMS or to medications used in the study (levetiracetam/lamotrigine/XEN1101). Experiment 1 was approved by King's College London Research Ethics Committee Research (CREC), which was performed in accordance with

relevant guidelines and regulations. Experiment 2 XEN1101 clinical trial [ClinicalTrials.gov Identifier: NCT03468725, registration date 02/03/2018)] was approved by the Medicines and Healthcare products Regulatory Agency (MHRA) in London. All participants signed a written informed consent before undergoing experimental procedures.

2.2 Experimental design and procedure

The experimental protocols and TEP modulation have already been published and they are described in more detail in previous reports [13,14]. In experiment 1, a single oral dose of lamotrigine (300 mg), a voltage-sensitive sodium channel blocker [19], or levetiracetam (3,000 mg), a specific binder of synaptic vesicle protein 2A (SV2A) [20], or placebo, were administered on separate occasions a week apart. In experiment 2, the novel selective positive allosteric modulator of potassium channel KCNQ2/3 (Kv7.2/7.3), XEN1101 (20 mg), in development for treatment of focal epilepsy by Xenon Pharmaceuticals Inc. [16], or placebo, were administered on separate occasions a week apart. Both studies followed a pseudo-randomized, double blinded, crossover design.

A TMS-compatible EEG system (BrainAmp MRplus; Brain Products) and Magstim 200^2 (Magstim Company Limited, Whitland, UK) TMS stimulator connected to a 90 mm figure-of-eight coil were used in both experiments. The EEG signal was acquired with a 64-electrode EasyCap for experiment 1 (EasyCap 64Ch; Brain Products) and a 64-electrode Multitrodes Cap (Multitrodes, BrainCap-Fast'n Easy) for experiment 2. An impedance below 10 kΩ was kept throughout the experiments during the recording.

At the beginning of each stimulation session, after identification of the first dorsal interosseous muscle (FDI) hotspot in the left M1, the resting motor threshold (RMT) was measured as the minimum intensity able to elicit a 50 μV peak-to-peak motor evoked potential in 5 out of 10 consecutive trials [21]. Using a 100% RMT intensity, 150 single pulses were delivered over the M1 hotspot. A masking noise was used to avoid possible contamination of the EEG signal by auditory potentials induced by the TMS click [22,23]. The same TMS intensity was used for baseline and post-drug stimulation, for two main reasons. The first reason was that, by adjusting the stimulation intensity to a new RMT value, possible TEP changes ascribable to the effects of AEDs would have

been confounded by effects of the different stimulation intensity. The second was to keep the artefact induced by scalp muscle activation by TMS constant, since it is known that the latter can partly contaminate TMS-EEG responses. However, the effects of AEDs on RMT were separately investigated by re-measuring RMT after drug intake [13,14].

TMS-EEG and resting EEG recordings were performed at baseline (pre-drug) and at 2 (experiment 1 and 2), 4 and 6 hours (experiment 2) after drug intake (figure 1). Five minutes before each post-drug measurement a blood sample was taken for every subject to evaluate drug plasma concentration. XEN1101 showed a pharmacokinetic profile characterized by a prolonged absorption and XEN1101 was detectable (<8.22 ng/mL) a week after administration, during the placebo experiment in those subjects who had placebo at the second visit [14]. Therefore, to investigate XEN1101 effects, we selected post-dose measures for TMS-EEG and resting EEG measurements taken during highest drug exposure (>8.22 ng/mL).

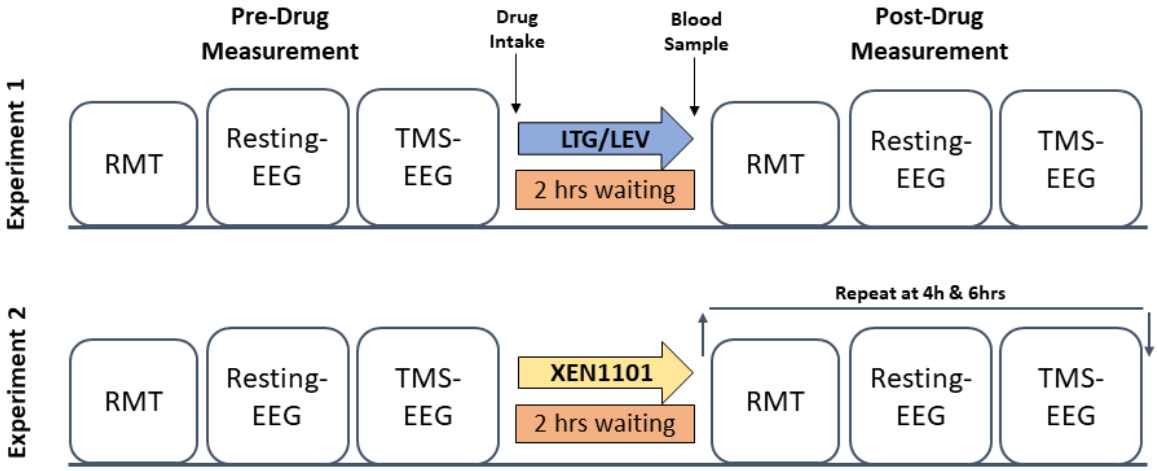

Figure 1: Experimental protocol and timeline.

Lamotrigine, levetiracetam and placebo were administered on separate occasions in experiment 1 and XEN1101 and placebo on separate occasions in experiment 2. Both studies followed a randomized and crossover design. RMT, Resting-state EEG and TMS-EEG sessions were recorded for each subject at baseline (pre-drug measurement) and at 2 (experiment 1 and 2), 4 and 6 hrs (experiment 2) after drug intake. Five minutes before each post-drug measurement a blood sample was taken to measure drug plasma concentration.

2.5 Analysis of TMS-induced oscillations

TMS-induced oscillations were analysed using MATLAB® (Mathworks Ltd, USA, R2012b) (The Mathworks Inc.) and FieldTrip toolbox [24]; the procedures have been described in detail elsewhere [17]. After excluding trials with prominent eye movements, blinks, and muscle artefacts (on the basis of visual inspection), data were down-sampled to 1kHz, segmented into epochs of 1 s length before and after the TMS pulse, and linearly interpolated for ±10 ms to remove the TMS artefact. Bad channels were removed from the EEG, and the signal was reconstructed by interpolating the surrounding electrode signals. Data were then notch-filtered (50 Hz). Independent Component analysis (ICA) was applied to remove TMS-related artifacts (i.e., cranial muscle response, recharge of capacitors, and related exponential decay artifacts [25-27]), as well as further muscle and ocular activity. Finally, remaining data were re-referenced to the linked mastoids, baseline corrected (from -1000 to -50 ms) and band-pass filtered (1-80 Hz).

Time-frequency (TF) representation was calculated by applying a Hanning taper windowed fast Fourier transform (FFT) with frequency-dependent window length (width: 3.5 cycles per time window, time steps: 10 ms, frequency steps: 1Hz from 2 to 45 Hz) [28]. TMS-induced responses were obtained by subtracting the individual time-domain average (ie. TEP) from each trial before calculating the TF of the single trials [29], therefore eliminating phase-locked activity which was not of interest in this study. Single-trial normalization by z-transforming the TF of each trial for each frequency was performed. The z-transformation was based on the respective mean and standard deviation derived from the full trial length. This was followed by an absolute baseline correction for each trial, by subtracting the average of the -1000 to -50 ms period for each frequency to ensure z-values represented a change from pre-TMS baseline [9,17]. For each drug condition in both experiments, TMS-induced oscillations were classified in delta (2-4 Hz), theta (4-8 Hz), alpha (8-12 Hz), beta (13-30 Hz) and gamma (30-45 Hz) frequency bands.

2.6 Analysis of resting-state EEG

To investigate the effects of the AEDs on spontaneous brain oscillations, 3 minutes of eyes closed resting state EEG data were analyzed. Data were segmented into 2 second epochs, band-pass filtered (2-80 Hz), down-sampled (1 kHz) and an automatic artefact rejection as implemented in Fieldtrip was conducted to remove epochs containing eye movements or muscle/movement

artifacts. Data were visually inspected, and epochs contaminated by residual artefacts were removed manually. The cleaned resting-state EEG data were then re-referenced to the average of all EEG channels. Power spectra were determined via FFT for frequency bins from 2 to 45 Hz in steps of 0.5 Hz, and spectra were averaged across segments and EEG channels. As for TMS-induced oscillation, resting-state EEG power was classified for discrete frequency bands, i.e. delta (2-4 Hz), theta (4-7 Hz), alpha (8-12 Hz), beta (13-30 Hz) and gamma (30-45 Hz) frequency bands.

3. Statistics

To investigate the effects of different AEDs on the power of TMS-induced oscillations and resting state EEG, multiple dependent sample t-tests at the individual electrode level within each drug condition (lamotrigine, levetiracetam and placebo in experiment 1; XEN1101 and placebo in experiment 2) were applied. Specifically, paired *t*-tests were applied to compare (i) post- versus pre-drug data within the same drug condition and (ii) between post-drug (or placebo) conditions, for each electrode in a selected a region of interest (ROI) that was composed of 27 channels around the stimulation site and the corresponding contralateral site (FC1, FC3, FC5, C1, C3, C5, CP1, CP3, CP5, P1, P3, P5, Cz, CPz, Pz, FC2, FC4, FC6, C2, C4, C6, CP2, CP4, CP6, P2, P4, P6) and for each frequency of interests: theta (4-7 Hz), alpha (8-12 Hz) beta (13-30 Hz) and gamma (30-45 Hz). TMS-induced oscillations were compared in a single time of interest from 30 to 800ms. Spectral fingerprints were corrected for multiple comparisons (i.e. electrodes, time and frequency points) and a cluster-based permutation analysis was applied (Maris & Oostenveld, 2007). T-values exceeding an a priori threshold of $p = 0.05$ were clustered based on adjacent time bins and neighboring electrodes. Cluster-level statistics was calculated by taking the sum of the t-values within every cluster and each statistical comparison was done with respect to the maximum values of summed t-values. Cluster-based permutation tests were performed as described above to check differences between pre-drug states within the same experiment, both for TMS-EEG and resting EEG signals, separately.

4. Results

For experiment 1 fifteen male subjects aged 19–34 years (mean age ± standard deviation [SD] 25.2 ± 4.62 years) were enrolled. Only one participant was unable to complete the TMS-EEG

procedure after the intake of lamotrigine due to side effects (i.e. vomiting), leaving a total number of 14 subjects[13]. Twenty subjects (mean age ± standard deviation (SD) of 26.6 ± 5.9 years (range 19–40 years) took part in experiment 2. XEN1101 showed a prolonged absorption and long elimination half-life, hence 4 participants did not reach XEN1101 concentrations higher than the carry-over observed in the placebo arm (8.2 ng/mL) at the time of TMS testing[14].

4.1 Antiepileptic drugs effects on TMS-induced oscillations

At baseline, prior to ingestion of drug or placebo, single-pulse TMS induced a specific pattern consisting of an early increase in power in the theta, alpha and beta bands; the latter showed a later decrease and a final increase in power, as described previously [9,10,17]. The baseline comparison of experiment 2 showed a higher TMS-induced increase in beta power from 560 to 710ms in the pre-placebo compared to pre-XEN1101 ($p=0.03$). Finally, placebo did not produce significant changes on the TMS-induced spectral profile in any frequency band and for both experiments (all p values > 0.05).

Lamotrigine

Compared to baseline, lamotrigine showed a significant increase in TMS-induced alpha band power ($p=0.01$, 340-490ms). This effect appears to be specifically located over channels close to the stimulated area (left M1) (figure 2). Comparisons between post-lamotrigine and post-placebo conditions supported the significant increase of the alpha band synchronization ($p=0.017$, 370-500ms).

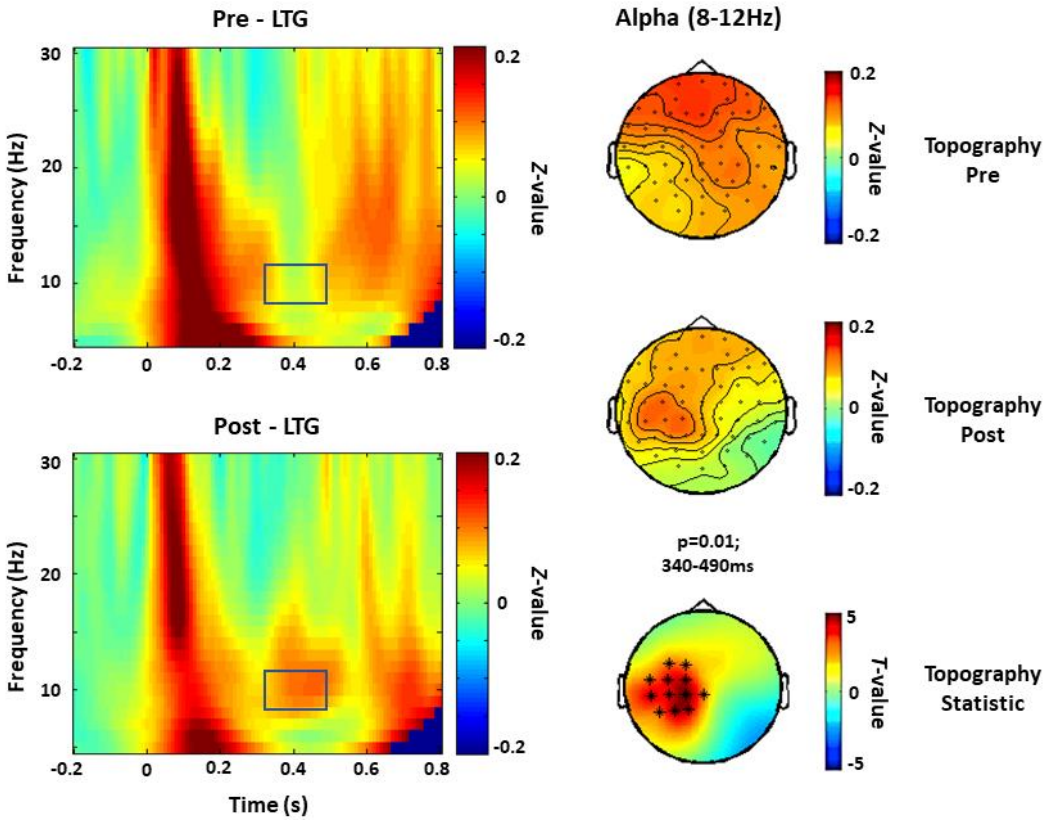

Figure 2: TMS-induced oscillations modulated by lamotrigine (experiment 1)

Grand averages of the time-frequency representation (TFR averaged over ROI channels) of TMS-induced oscillations recorded before (pre) and after (post) the intake of lamotrigine are shown on the left panel. The blue boxes correspond to the time window when comparison between pre and post conditions showed significant drug effects. Topographical distributions of drug-induced effects on the alpha band power (averaged over 340-390ms post TMS; p=0.01,) are reported for pre and post drug conditions on the right panel. Significant electrodes within the bilateral 27 electrode ROIs are represented with asterisks in the t-statistic map.

Levetiracetam

As shown in figure 3, levetiracetam induced a reduction in TMS-induced theta (p=0.001, 30-310ms) and alpha (p=0.01, 80-300ms), early beta (p=0.01, 70-160ms) and late beta (p=0.03, 550-670ms) power. This occurred in a large area, including bilateral central and parietal electrodes.

The comparison between post-levetiracetam and post-placebo confirmed the suppression of theta (p=0.006, 110-350ms) and alpha (p=0.004, 90-340ms) power, whereas the effects on the beta band were not significant (p>0.05).

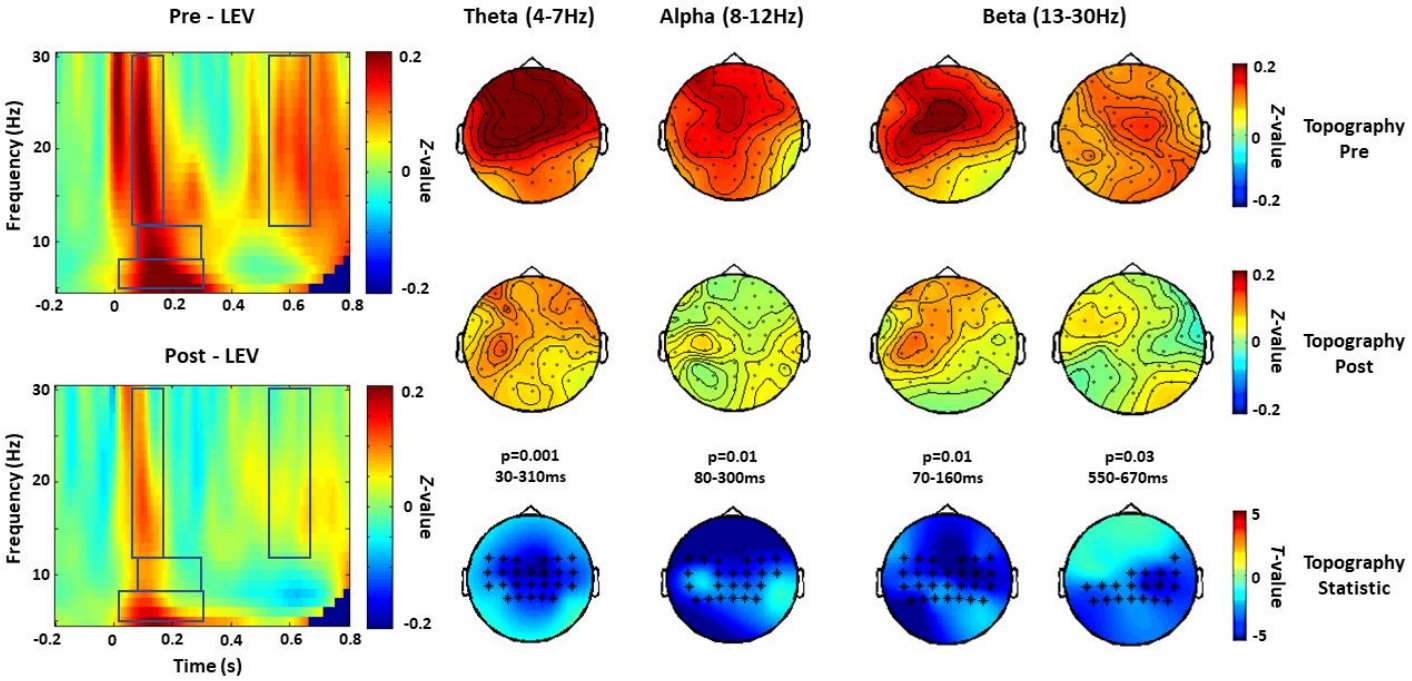

Figure 3: TMS-induced oscillations modulated by levetiracetam (experiment 1)

Grand averages of the time-frequency representation (TFR averaged over ROI channels) of TMS-induced oscillations recorded before (pre) and after (post) the intake of levetiracetam are shown on the left panel. The blue boxes correspond to the time and frequency windows when comparisons between pre and post conditions showed significant drug effects. Topographical distribution of drug-induced effects on the theta (p=0.001, 30-310ms), alpha (p=0.01, 80-300ms) and beta (p=0.01, 70-160ms; p=0.03, 550-670ms) band power are reported for pre and post drug conditions on the right panel. Significant electrodes within the bilateral 27 electrode ROIs are represented with asterisks in the t-statistic maps.

XEN1101

XEN1101 induced a significant suppression of delta power over electrodes close to the stimulated area (p=0.05, 100-160ms), a significant decrease of theta power over stimulated and contralateral channels (p=0.02, 30-370ms) and a decrease in TMS-induced beta power in the right hemisphere (p=0.04, 210-300ms) (Figure 4). The effect on beta band power was supported by the comparison against post-placebo (p=0.03, significant effects from 30 to 400ms). The other comparisons did not show significant effect of this drug on the late beta and alpha powers (p>0.05).

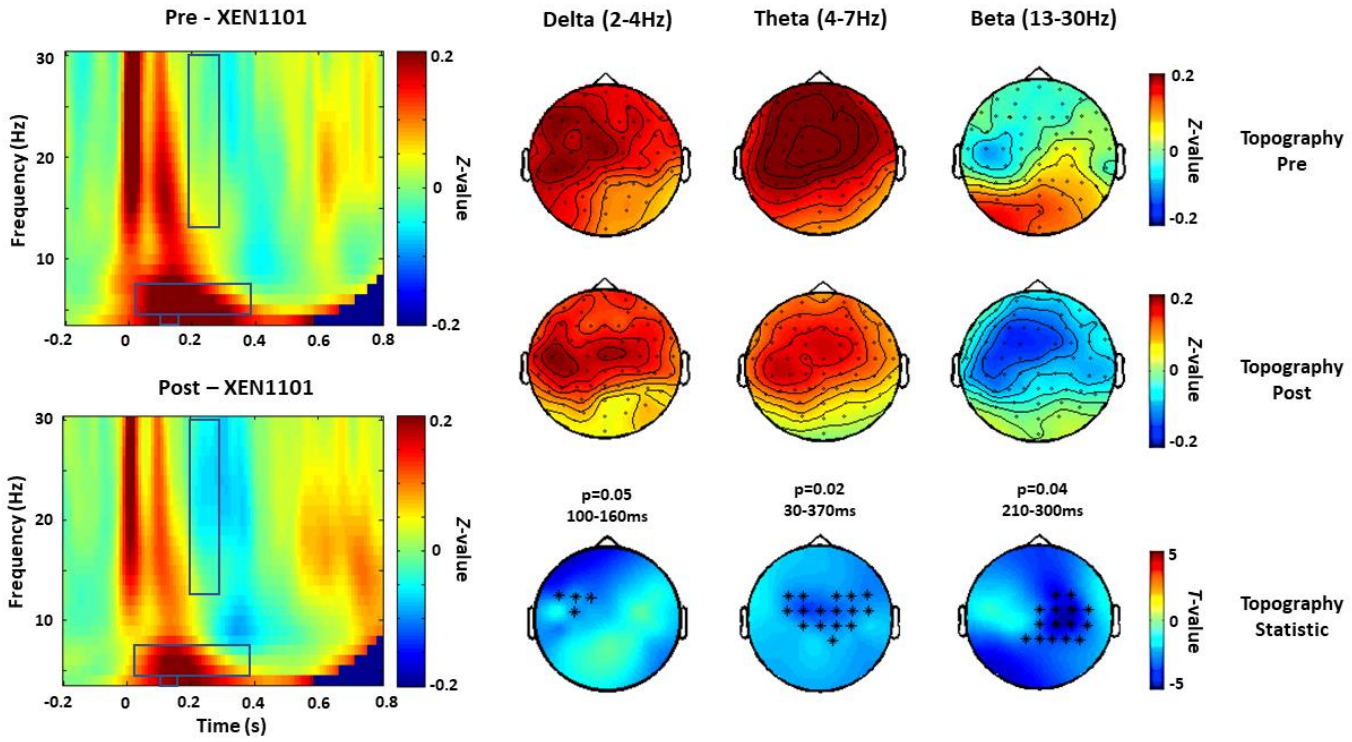

Figure 4: TMS-induced oscillations modulated by XEN1101 (experiment 2)

Grand averages of the time-frequency representation (TFR averaged over ROI channels) of TMS-induced oscillations recorded before (pre) and after (post) the intake of XEN1101 are shown on the left panel. The blue boxes correspond to the time and frequency windows when comparisons between pre and post conditions showed significant drug effects. Topographical distribution of drug-induced effects on the delta (p=0.05, 100-160), theta (p=0.02, 30-370ms) and beta (p=0.04, 210-300ms) band power are reported for pre and post drug conditions on the right panel. Significant electrodes within the bilateral 27 electrode ROIs are represented with asterisks in the t-statistic maps.

4.2 Antiepileptic drugs effects on Resting State EEG

In both experiments, the cluster-based permutation comparisons between pre-drug or pre-placebo conditions did not show significant differences in the resting oscillatory power in all frequency bands (all p>0.05). Finally, placebo did not produce significant changes on resting-state EEG spectral profile in any frequency band and for both experiments (all p>0.05).

Lamotrigine

Compared to pre-drug intake, lamotrigine significantly decreased spontaneous theta band power (p=0.03) over all sensors compared and had no effect on other frequency bands (p>0.05; Figure

5A). The same significant modulation was confirmed when comparing post-lamotrigine versus post-placebo ($p=0.01$).

Levetiracetam

Compared to baseline, a single dose of levetiracetam significantly enhanced beta ($p<0.001$) and gamma power ($p=0.001$) over medial frontal and parietal electrodes and theta band power at a right lateral cluster ($p=0.03$; Figure 5B). The modulatory effect of levetiracetam on beta and gamma band oscillations was supported by the comparison with the post-placebo condition ($p=0.03$ and $p=0.04$); however, differences on theta and alpha bands were not significant ($p>0.05$).

XEN1101

In subjects showing good XEN1101 exposure (plasma levels >8.22ng/mL) at the time of assessments, resting state oscillatory activity was significantly modulated. Specifically, a significant increase in delta frequency power ($p<0.001$) in frontal, parietal and occipital electrodes, a significant power increase of theta and beta bands in medial frontal and parietal electrodes ($p=0.01$ and $p=0.005$) and an increase in gamma power ($p=0.02$) in the occipital electrodes were found (Figure 5C). All these effects were confirmed when XEN1101 was compared with the post-placebo condition. The analysis showed an enhanced power of delta ($p<0.001$), theta ($p=0.04$), beta ($p<0.01$) and gamma ($p=0.02$) frequency bands.

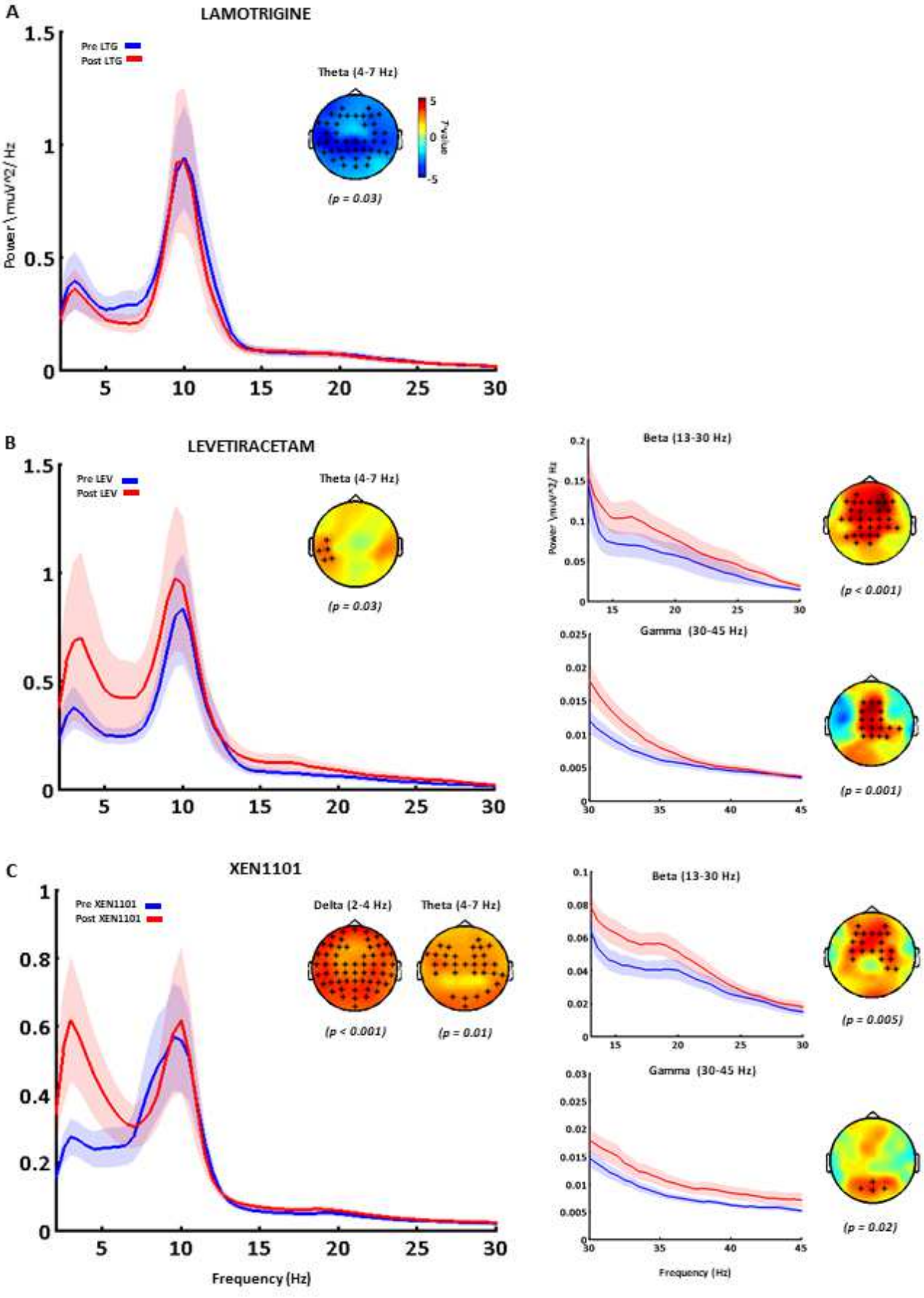
A
LAMOTRIGINE
Pre LTG
Post LTG
Power \ muV^2 / Hz
Theta (4-7 Hz)
T-value
(p = 0.03)
B
LEVETIRACETAM
Pre LEV
Post LEV
Theta (4-7 Hz)
(p = 0.03)
Beta (13-30 Hz)
(p < 0.001)
Gamma (30-45 Hz)
(p = 0.001)
C
XEN1101
Pre XEN1101
Post XEN1101
Delta (2-4 Hz)
Theta (4-7 Hz)
(p < 0.001)
(p = 0.01)
Beta (13-30 Hz)
(p = 0.005)
Gamma (30-45 Hz)
(p = 0.02)
Frequency (Hz)

Figure 5: The effects of antiepileptic drugs on resting-state EEG oscillations

Grand-averaged power spectrums calculated on the average of all channels are reported before (pre, blue) and after (post, red) the intake of lamotrigine (a), levetiracetam (b) and XEN1101 (c). For each drug condition, significant differences are indicated with the respective topographical distribution of t-values where significant channels are indicated with asterisks. Lamotrigine (a) decreases theta power ($p=0.03$); Levetiracetam (b) increases theta ($p=0.03$), beta ($p<0.001$) and gamma ($p=0.001$) power; XEN1101 increases delta ($p<0.001$), theta ($p=0.01$), beta ($p=0.005$) and gamma ($p=0.02$) power. The significant modulation of beta and gamma power are shown for each drug in a zoomed power spectrum (panels on the right; averaged over significant channels for levetiracetam and XEN1101, respectively).

5. Discussion

Our results extend previous studies where we demonstrated that non-invasive electrodiagnostic techniques, such as TMS-EEG, can inform our understanding of mechanisms of action of drugs acting on the CNS. Here we investigated the modulation of TMS-induced cortical oscillations and resting-state EEG power by AEDs with different mechanisms of action in healthy volunteers.

In the TMS-induced oscillation analysis, delta power was decreased only by XEN1101, theta was suppressed by levetiracetam and XEN1101, whereas alpha power was suppressed by levetiracetam and increased by lamotrigine. Finally, beta power was reduced by levetiracetam in two time windows (70-160ms and 550-670ms), and it was reduced by XEN1101 from 210 to 300ms where typically beta is suppressed (or de-synchronized) by TMS [17]. Table 1 summarise these new results, as well as others available in literature. Signatures at the resting state EEG level showed a common pattern for l and XEN1101 with an increase of theta, beta and gamma power in contrast with suppression of theta power by lamotrigine. Finally, delta power was increased by XEN1101 only.

Drugs	Mechanism of Action	Dose	Delta	Theta	Alpha	Beta	Gamma
Lamotrigine	VGSC blocker	300mg	n.s.	n.s.	↑	n.s.	n.s.
Levetiracetam	Inhibitor of excitatory NT release	3000mg	n.s.	↓	↓	↓	n.s.
XEN1101	Modulator of the potassium channel KCNQ2/3	20mg	↓	↓	n.s.	↓	n.s.
Alprazolam[17]	GABA-AR agonist	1mg	n.p.	n.p.	n.s.	↓	n.p.
Baclofen[17]	GABA-BR agonist	50mg	n.p.	n.p.	↓	↓	n.p.
Diazepam[17]	GABA-AR agonist	20mg	n.p.	n.p.	↑	↓	n.p.
Zolpidem[17]	GABA-AR agonist	10mg	n.p.	n.p.	↑	n.s.	n.p.

Table 1. Summary of TMS-induced oscillations modulated by CNS active drugs
n.p. = not perfromed; n.s. = non significant; ↑ = increase of power; ↓ = decrease of power.

5.1 Cortical oscillations to study neural processes

Cortical oscillations have been widely studied in the EEG literature and specific frequency bands have been associated with distinct behaviours or cognitive functions [30,31]; their pattern may also reflect dysfunction in neural networks in a pathological brain [32]. As we showed in our work, cortical oscillations can be observed at rest, when no task is performed, or they can be induced by a given stimulus (i.e., TMS).

5.2 TMS-induced oscillations to investigate the effects of antiepileptic drugs

Changes in cortical oscillations following TMS on M1 involve an increase in power of delta, theta, alpha and beta frequency bands in a period up to 300ms after TMS, followed by a beta reduction (de-synchronization) and a subsequent beta increase [10]; despite their reproducibility, the mechanisms underlying this phenomena are still not fully understood. Pharmacological investigations suggested that early power increase (up to 200ms) and late decrease (200-400ms)

of induced oscillations may be mediated by separate inhibitory mechanisms. As such, early increase in alpha-band power was enhanced by a GABAAR-mediated drive (zolpidem, diazepam and alprazolam) and reduced by GABABR-mediated activity (baclofen), whereas both GABAAR- and GABABR-activity enhanced the reduction of beta-band power. Finally, the GABABR agonist baclofen enhanced the reduction of alpha-band power [17] (Table1).

In the light of this existing literature we may speculate that the increase of the TMS-induced alpha oscillation by lamotrigine is in line with the effects of diazepam and zolpidem [17] and this may suggest an effect mediated by local cortical circuits in which GABA-A synapses have a predominant effect. Lamotrigine, which is also used as a mood stabilizer, does not act directly on GABAAR; however, it was reported to increase the amplitude and the frequency of spontaneous GABAAR-mediated inhibitory postsynaptic currents by increasing GABA release in vitro [33]. In TMS-EEG experiments, lamotrigine increased the N45 TEP component [13], which is related to GABAergic neurotransmission [11].

Levetiracetam and XEN1101 show a similar pattern of suppression of TMS-induced theta activity up to ~300ms. During the same time window, levetiracetam extends the suppression also over alpha and beta band power, whereas at 550-670ms levetiracetam reduces beta power over the rebound period and XEN1101 reduces beta power at 210-300ms exactly as baclofen, diazepam and alprazolam. The exact mechanisms driving the TMS-induced de-synchronization/rebound of beta oscillations over sensorimotor cortices is not well understood; however, it may be a direct consequence of the transcranial activation of beta oscillation generating cortico-cortical and cortico-subcortical circuits. The hypothesis that the motor thalamus facilitates the cortically-generated TMS-induced beta oscillations through cortico-subcortico-cortical feedback loops is supported by a study conducted on a patient with Parkinson's disease who had undergone unilateral surgical lesioning of the ventrolateral nucleus of the thalamus [34]. The beta oscillation obtained in response to pulses applied over the intact hemisphere was higher than that obtained in healthy controls. The authors proposed that the thalamotomy served to reduce the abnormally high TMS-induced beta oscillations [34]. Another study demonstrated that patients with severe disorders of consciousness failed to show TMS induced alpha and beta desynchronization [35]. This pattern may reflect a consequence of the breakdown of cortico-cortical neuronal connectivity.

Interestingly, TMS-evoked oscillations at the motor area at longer time intervals (400–700ms) were abnormally increased in patients with schizophrenia [36], suggesting a possible disinhibition of the motor cortex.

It is important to highlight that TMS-evoked EEG potentials were modulated in a similar way by lamotrigine and levetiracetam. To further explore this finding, it is useful to refer to a recently developed computational approach which enables the analysis of high-dimensional datasets, to reveal low-dimensional descriptions of effects [37]. A relatively simple model (PARAFAC) was validated to show the joint effect of levetiracetam and lamotrigine over channels, time, frequency, subjects and in comparison with pre-drug intake. The model revealed that both drugs suppress oscillations in the alpha range in the occipital region and that this effect was stronger with levetiracetam. These results demonstrate that time-frequency decomposition may reveal additional relevant features of AEDs effects.

5.3 Spontaneous cortical oscillations to investigate the effects of antiepileptic drugs

Several studies have attempted to address candidate mechanisms of resting-state oscillation generation; for example, some pyramidal neurons in the visual cortex have been shown to engage in spontaneous rhythmic firing due to their intrinsic membrane properties [38]. Oscillations can also occur in loops involving the thalamus, other cortical areas, subcortical structures, or the spinal cord. Finally, a number of studies have shown that an isolated cortical network can produce stable oscillatory discharges and that inhibitory interneurons play the critical orchestrator role by periodically silencing bursts from excitatory cells [39,40]. Given that benzodiazepines act on GABA-A receptors to increase inhibitory post-synaptic potentials (IPSPs), they have been used to explore the underlying mechanism and functional role of cortical oscillations. It is well known in clinical practice that, at rest, benzodiazepines enhance beta band power in EEG recorded from the frontal cortex [41], whereas they often reduce α-band power (usually reported for parieto-occipital regions) [42,43]. Baclofen, a GABA-B receptor agonist which mediates inhibition by increasing K^+ currents, induces an increase in spontaneous alpha and beta power [17]. Theta oscillations were first discovered in the rabbit hippocampus in 1938 [44]. The theta-memory link was later specifically strengthened by studies showing that the phase of theta

oscillations modulates synaptic plasticity [45]. Finally, gamma oscillations have been extensively investigated given their influence of cognition and abnormal behaviour in schizophrenia [46].

Resting state EEG recording has been applied together with AEDs in a series of studies to describe quantitative changes of EEG signals. The effects of lamotrigine and levetiracetam have been tested in healthy participants and results show a decrease of theta and alpha spectral power for lamotrigine [47] and no significant modulation for levetiracetam [48]. The discrepancy with our results for levetiracetam may result from the EEG acquisition system which used a lower number of electrodes than in our experiment here. However, in patients with epilepsy, levetiracetam showed a consistent increase of relative beta power [49,50]. Changes in theta and beta bands have been correlated to improved performance in cognitive tests for attention, working memory and executive functions [50]. As an example, in patients with Alzheimer's disease, levetiracetam produces a reduction in power of low frequency bands (delta) and an increase of beta bands [51]. Similar effects have been observed for the first time with XEN1101. The increase of beta waves particularly in the frontal areas has already been reported in the literature after benzodiazepines intake [41].

Levetiracetam and XEN1101 increased gamma frequency power, levetiracetam over centro-frontal sites, whereas XEN1101 on occipital channels. High-frequency power increase is likely generated through networks in a cycle of GABA-A receptors-mediated alternating inhibition and excitation [52]. Excitatory NMDA receptors contribute to the generation of network oscillations via modulation of both interneuron to interneuron and interneuron to pyramidal neuron transmission. These oscillations are controlled by a specific class of inhibitory interneurons that can be identified based on either their fast-spiking electrophysiological properties or their expression of calcium-binding protein parvalbumin (PV) [53,54]. Kv7.2 and Kv7.3 channels are expressed in regular and fast-spiking interneurons and retigabine, a K^+ channel opener with similar properties to XEN1101, showed effects on these neural elements [55]. Therefore, we may speculate that XEN1101 potentiates K^+ currents over interneurons generating activity in the gamma frequency band.

Levetiracetam has been demonstrated to modulate cognitive functions and this effect has been related to theta oscillations [56]; however, there are no published results on gamma oscillations. The gamma power increase induced by levetiracetam speaks in favour of a possible impact on cognitive functions, given the association between higher gamma synchrony with stronger neural network engagement through establishing correlations with specific functions [46].

5.4 Limitations

An important limitation is the difficulty to mechanistically interpret our results, due to a lack of similar studies in the literature**.** We believe that future pharmaco-EEG studies should focus their analysis on brain oscillations, to develop a comprehensive database of AED effects on this biomarker. Finally, TMS was delivered at 100% RMT intensity which may add potential confounds to the real cortical TMS-EEG response given by auditory and somatosensory activities [57]. However, it was recently shown that for M1 it is possible to obtain genuine brain responses up to 300 ms with the implementation of careful control of confounding sources [57].

5.5 Conclusion

In conclusion, we showed that measuring brain oscillations, either spontaneous or induced by TMS, may have a great potential as a biomarker of mechanisms of action in studies assessing the effect of AEDs. The implementation of new biomarkers will allow to accelerate early development of new drugs, by revealing that a new drug acts on and modulates a target mechanism of interest, with possible positive implications for patients' quality of life, health care providers and pharmaceutical companies. For all these reasons, it is crucial to develop a more thorough and systematic account of changes in brain oscillations induced by AEDs. In addition, these results will provide further insight to pathophysiology of other neurological and psychiatric conditions and to the evaluation of neurophysiological basis of cognition.

6. Acknowledgements:

MPR is funded by MRC Programme Grant MR/K013998/1, EPSRC Centre for Predictive Modelling in Healthcare EP/N014391/1, and by the NIHR Biomedical Research Centre and South London and Maudsley NHS Foundation Trust and King's College London. IP is partly funded by Xenon

Pharmaceuticals Inc. This study represents independent research supported by the National Institute for Health Research (NIHR)-Well come King's Clinical Research Facility and the NIHR Biomedical Research Centre at South London and Maudsley NHS Foundation Trust and King's College London. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health and Social Care.

7. Conflict of Interest:

The author GNB declares a conflict of interest, as an employee of Xenon Pharmaceuticals. Inc. Burnaby Canada (https://www.xenon-pharma.com/) and having been granted incentive stock options in the company. The other authors have not conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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Figures

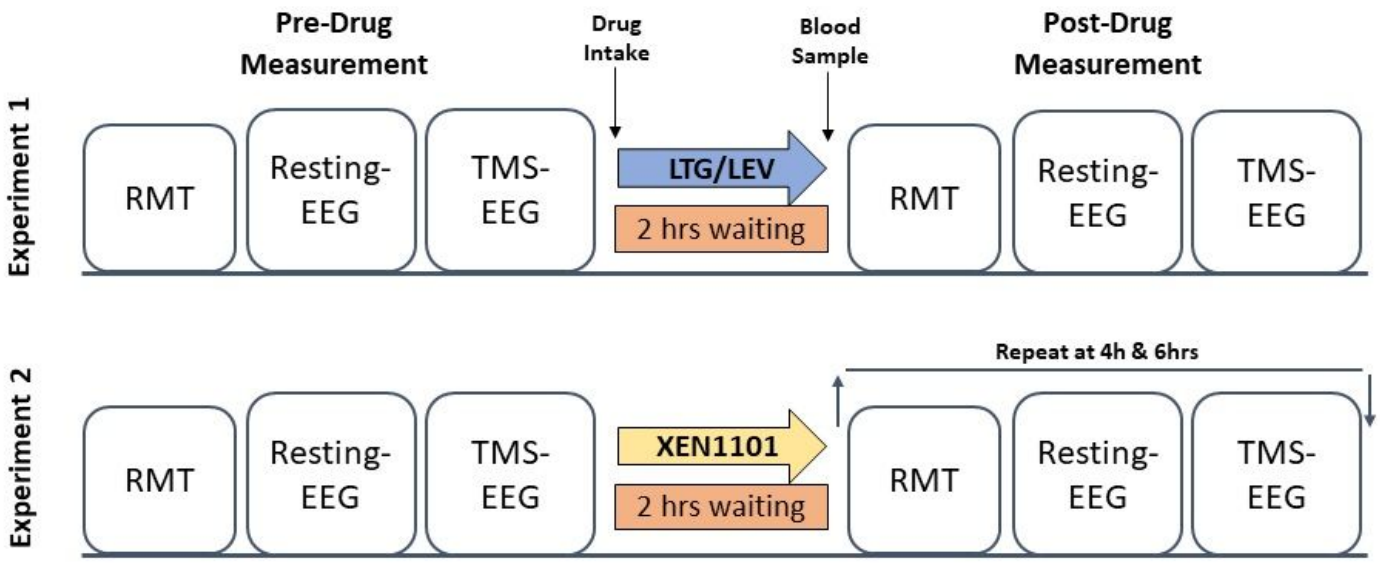

Figure 1

Experimental protocol and timeline. Lamotrigine, levetiracetam and placebo were administered on separate occasions in experiment 1 and XEN1101 and placebo on separate occasions in experiment 2. Both studies followed a randomized and crossover design. RMT, Resting-state EEG and TMS-EEG sessions were recorded for each subject at baseline (pre-drug measurement) and at 2 (experiment 1 and 2), 4 and 6 hrs (experiment 2) after drug intake. Five minutes before each post-drug measurement a blood sample was taken to measure drug plasma concentration.

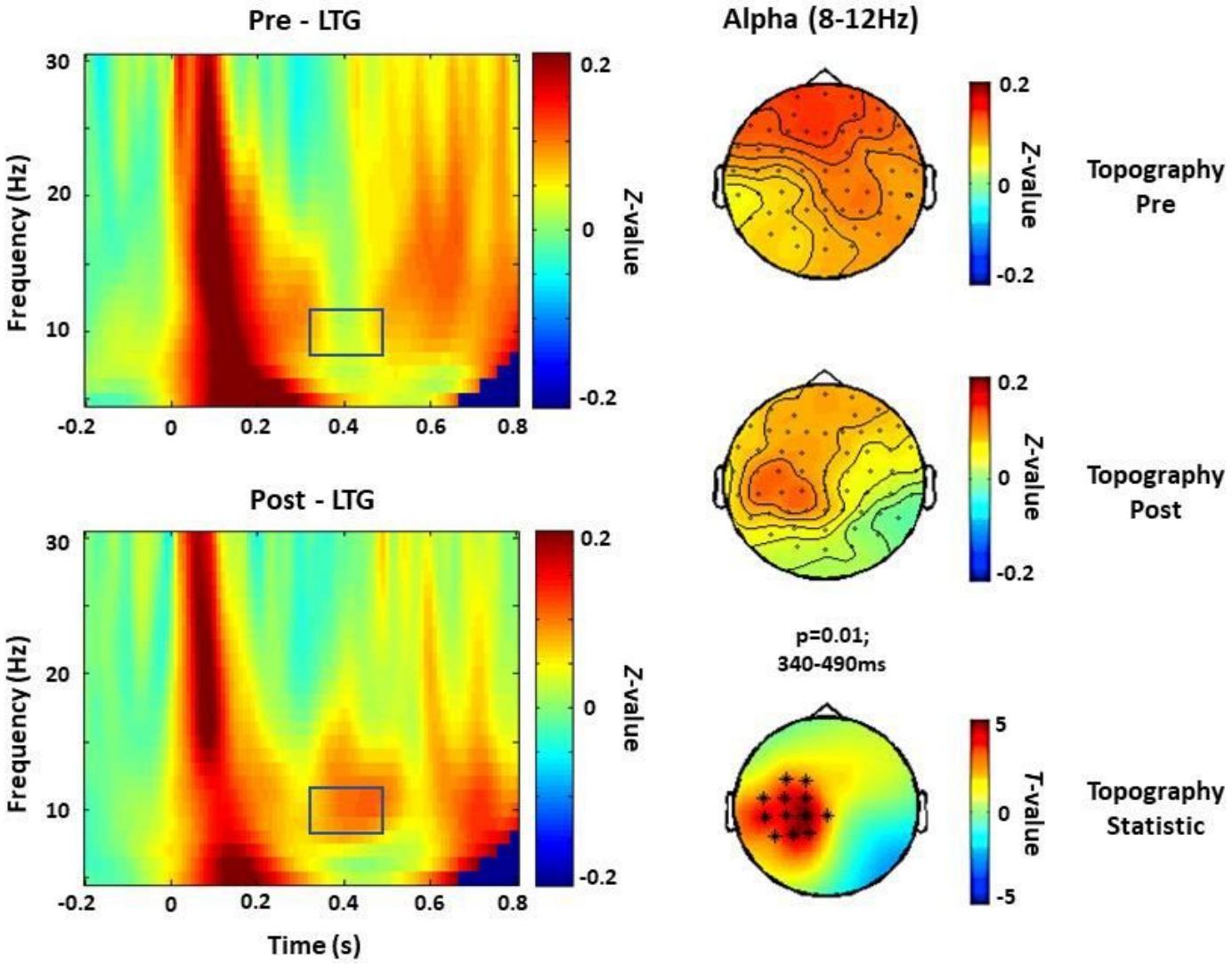

Figure 2

TMS-induced oscillations modulated by lamotrigine (experiment 1) Grand averages of the time-frequency representation (TFR averaged over ROI channels) of TMS-induced oscillations recorded before (pre) and after (post) the intake of lamotrigine are shown on the left panel. The blue boxes correspond to the time window when comparison between pre and post conditions showed significant drug effects. Topographical distributions of drug-induced effects on the alpha band power (averaged over 340-390ms post TMS; p=0.01,) are reported for pre and post drug conditions on the right panel. Significant electrodes within the bilateral 27 electrode ROIs are represented with asterisks in the t-statistic map.

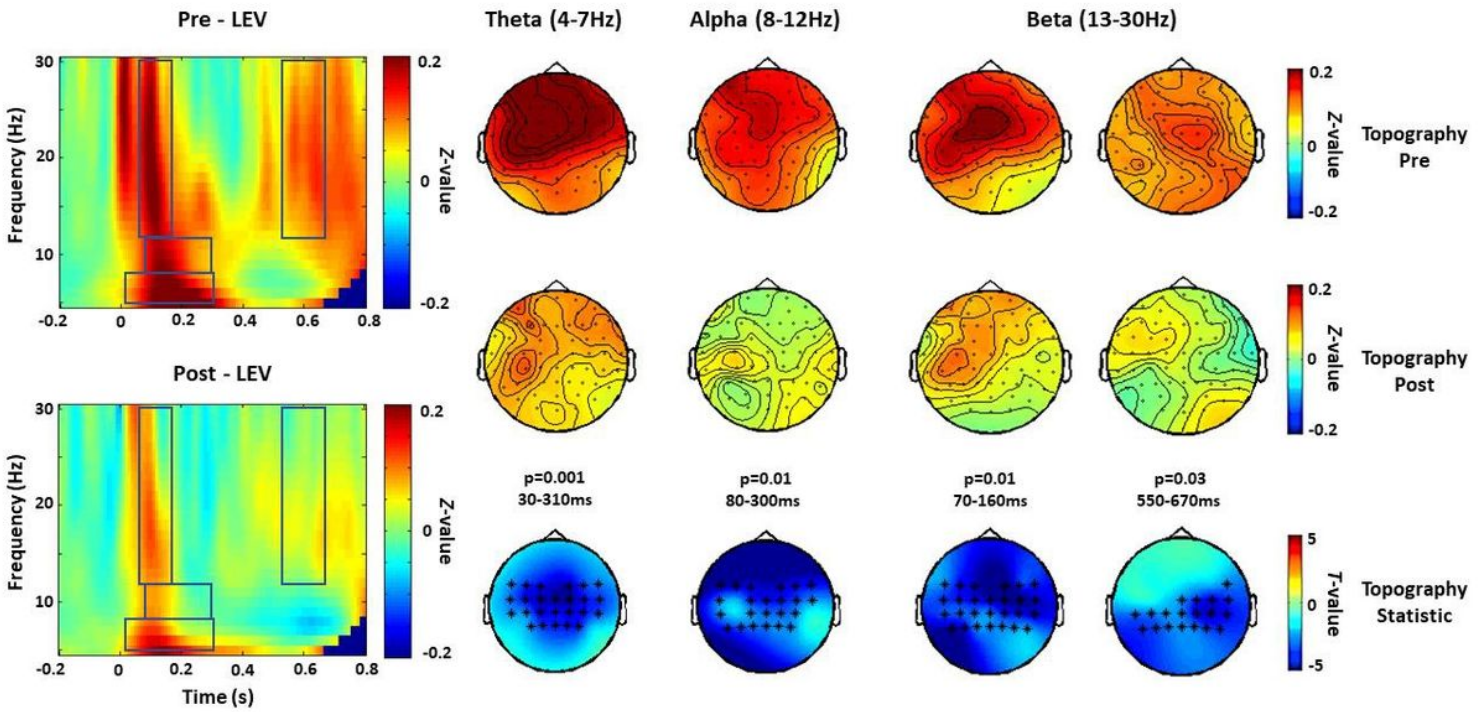

Figure 3

TMS-induced oscillations modulated by levetiracetam (experiment 1) Grand averages of the time-frequency representation (TFR averaged over ROI channels) of TMS-induced oscillations recorded before (pre) and after (post) the intake of levetiracetam are shown on the left panel. The blue boxes correspond to the time and frequency windows when comparisons between pre and post conditions showed significant drug effects. Topographical distribution of drug-induced effects on the theta (p=0.001, 30-310ms), alpha (p=0.01, 80-300ms) and beta (p=0.01, 70-160ms; p=0.03, 550-670ms) band power are reported for pre and post drug conditions on the right panel. Significant electrodes within the bilateral 27 electrode ROIs are represented with asterisks in the t-statistic maps.

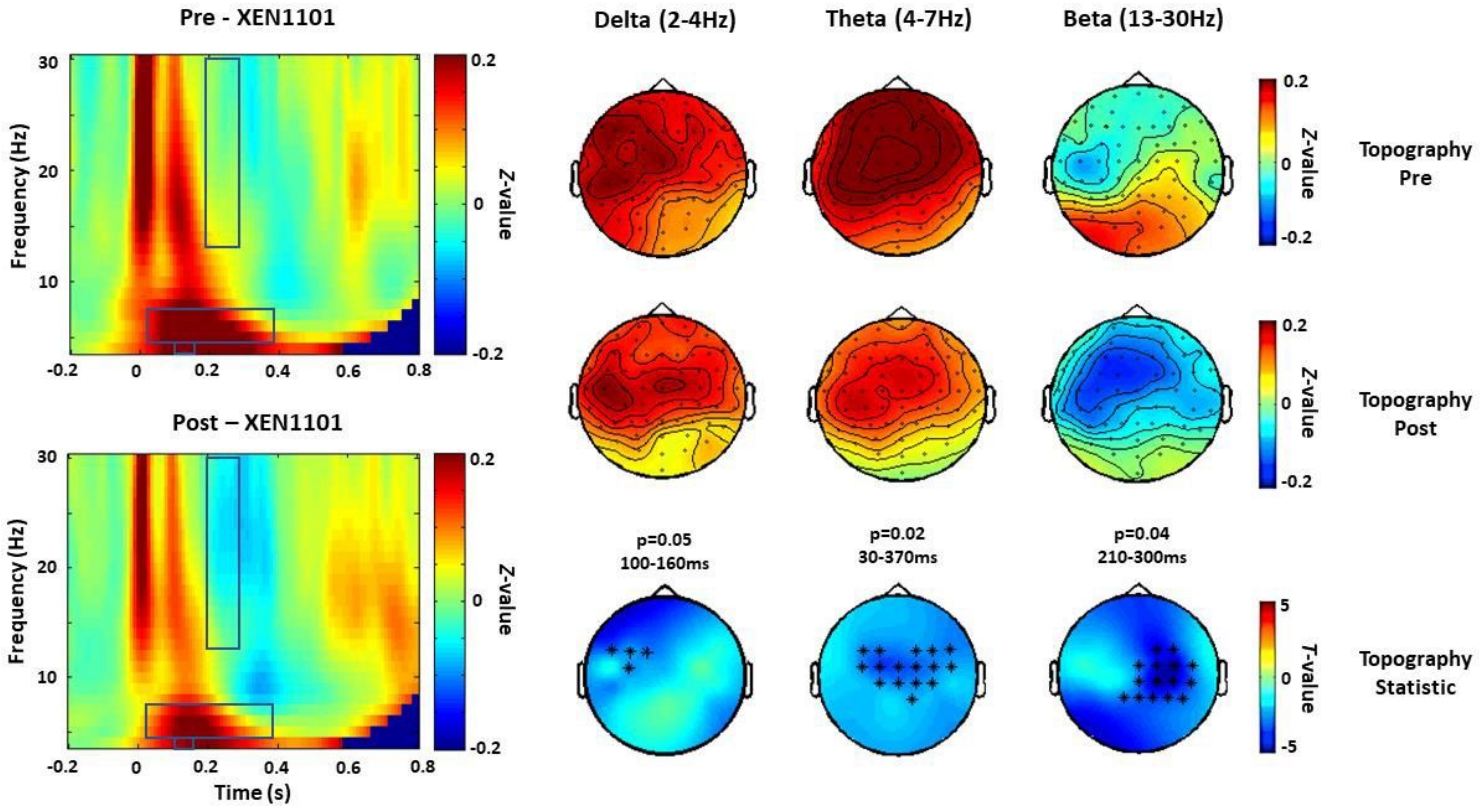

Figure 4

TMS-induced oscillations modulated by XEN1101 (experiment 2) Grand averages of the time-frequency representation (TFR averaged over ROI channels) of TMS-induced oscillations recorded before (pre) and after (post) the intake of XEN1101 are shown on the left panel. The blue boxes correspond to the time and frequency windows when comparisons between pre and post conditions showed significant drug effects. Topographical distribution of drug-induced effects on the delta (p=0.05, 100-160), theta (p=0.02, 30-370ms) and beta (p=0.04, 210-300ms) band power are reported for pre and post drug conditions on the right panel. Significant electrodes within the bilateral 27 electrode ROIs are represented with asterisks in the t-statistic maps.

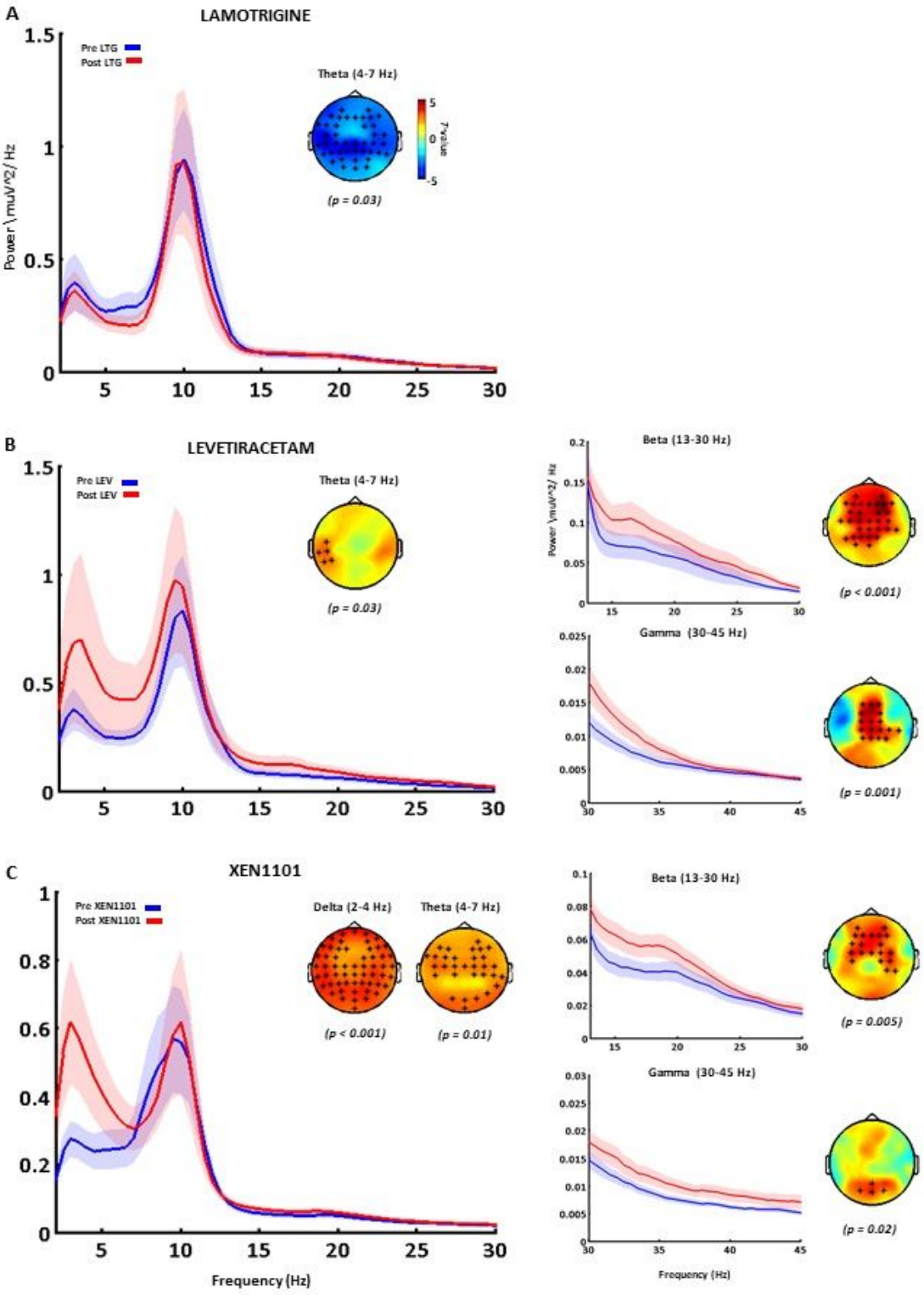

Figure 5

The effects of antiepileptic drugs on resting-state EEG oscillations Grand-averaged power spectrums calculated on the average of all channels are reported before (pre, blue) and after (post, red) the intake of lamotrigine (a), levetiracetam (b) and XEN1101 (c). For each drug condition, significant differences are indicated with the respective topographical distribution of t-values where significant channels are indicated with asterisks. Lamotrigine (a) decreases theta power (p=0.03); Levetiracetam (b) increases

theta ($p=0.03$), beta ($p<0.001$) and gamma ($p=0.001$) power; XEN1101 increases delta ($p<0.001$), theta ($p=0.01$), beta ($p=0.005$) and gamma ($p=0.02$) power. The significant modulation of beta and gamma power are shown for each drug in a zoomed power spectrum (panels on the right; averaged over significant channels for levetiracetam and XEN1101, respectively).