Insomnia disorder and endogenous neurophysiological dynamics
Colombo, M.

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Chapter 2

Wake high-density Electroencephalographic spatiotemporal signatures of insomnia

Authors
Michele A. Colombo1,4,5, MSc
Jennifer R. Ramautar1, PhD
Yishul Wei1, MSc
Germán Gomez-Herrero1, PhD
Diederick Stoffers1, PhD
Rick Wassing1, MSc
Jeroen S. Benjamins1,6, PhD
Enzo Tagliazucchi1, PhD
Ysbrand D. van der Werf1,2, PhD
Christian Cajochen5, PhD
Eus J. W. Van Someren1,3, PhD

Affiliations
1 Department of Sleep and Cognition, Netherlands Institute for Neuroscience (NIN), an institute of the Royal Netherlands Academy of Arts and Sciences, Amsterdam, the Netherlands;
2 Department of Anatomy and Neurosciences, VU University Medical Center, Amsterdam, the Netherlands;
3 Department of Integrative Neurophysiology and Medical Psychology, Center for Neurogenomics and Cognitive Research (CNCR), Neuroscience Campus Amsterdam, VU University and Medical Center, Amsterdam, the Netherlands;
4 Faculty of Biology, and Bernstein Center Freiburg, University of Freiburg, Freiburg, Germany;
5 Centre for Chronobiology, Psychiatric Hospital of the University of Basel (UPK), Basel, Switzerland
6 Department of Clinical and Health Psychology, Department of Experimental Psychology, Utrecht University, Heidelberglaan 1, Utrecht, 3584 CS, The Netherlands

Contributions
MC, JR, YW, GG-H, DS, RW, JB, YvdW contributed to data collection; MC, GG-H, YW, ET performed the analysis; MC wrote the manuscript; JR set up the laboratory for data collection; GG-H, DS set-up the computational resources on the server; JR, YW, GG-H, ET provided fruitful interpretation of the data; YvdW, CC, EVS oversaw the project; EVS designed the data acquisition protocol; All authors participated in the revision of the manuscript.

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Wake High-Density Electroencephalographic Spatiopectral Signatures of Insomnia

Abstract

Study Objectives
Although daytime complaints are a defining characteristic of insomnia, most EEG studies evaluated sleep only. We used high-density electroencephalography to investigate wake resting state oscillations characteristic of insomnia disorder (ID) at a fine-grained spatiopectral resolution.

Methods
A case-control assessment during eyes open (EO) and eyes closed (EC) was performed in a laboratory for human physiology. Participants (n = 94, 74 female, 21–70 y) were recruited through www.sleepregistry.nl: 51 with ID, according to DSM-5 and 43 matched controls. Exclusion criteria were any somatic, neurological or psychiatric condition. Group differences in the spectral power topographies across multiple frequencies (1.5 to 40 Hz) were evaluated using permutation-based inference with Threshold-Free Cluster-Enhancement, to correct for multiple comparisons.

Results
As compared to controls, participants with ID showed less power in a narrow upper alpha band (11–12.7 Hz, peak: 11.7 Hz) over bilateral frontal and left temporal regions during EO, and more power in a broad beta frequency range (16.3–40 Hz, peak: 19 Hz) globally during EC. Source estimates suggested global rather than cortically localized group differences.

Conclusions
The widespread high power in a broad beta band reported previously during sleep in insomnia is present as well during eyes closed wakefulness, suggestive of a round-the-clock hyperarousal. Low power in the upper alpha band during eyes open is consistent with low cortical inhibition and attentional filtering. The fine-grained HD-EEG findings suggest that, while more feasible than PSG, wake EEG of short duration with a few well-chosen electrodes and frequency bands, can provide valuable features of insomnia.

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1. Introduction

Insomnia is characterized by poor sleep quality including problems initiating or maintaining sleep, early morning awakening and nonrestorative sleep (American Psychiatric Association, 2013). It is accompanied by daytime repercussions (American Psychiatric Association, 2013; Ustinov et al., 2010) on mood (Espie et al., 2012), energy, and memory (Shekleton et al., 2014) and is among the most common health complaints (Ohayon, 2002), affecting around 6% of the adult population in its chronic form. Chronic insomnia carries an increased risk of severe, long-lasting health problems, including cardiovascular (Laugsand et al., 2011; Spiegelhalder et al., 2011) and mood (Baglioni et al., 2011) disorders.

Generalized hyperarousal plays a key role in the pathway of the cognitive and behavioral factors leading to chronic insomnia (Riemann et al., 2010, 2015), according to recent developments of the neurocognitive model (Bonnet and Arand, 2010b; Perlis et al., 1997, 2001). Evidence of hyperarousal has been found across the sleep-wake cycle (Bonnet and Arand, 1997, 2010b) and in the somatic, cognitive and neurobiological domains (Perlis et al., 2001), leading to the hypothesis that insomnia is maintained by a continuous, multi-component hyperarousal. Hyperarousal of the central nervous system may result from an imbalance between wake and sleep promoting systems, but could also arise from other structural and functional deviations in emotion and reward regulation systems, interfering with the normally cycling expression of sleep and wakefulness (Romeijn et al., 2012). Local reductions in gray matter volume in the orbitofrontal cortex have been reported in association with insomnia (Altena et al., 2010), with sleep fragmentation (Lim et al., 2015) and with the vulnerability for early morning awakening (Stoffers et al., 2012). Given the role of the orbitofrontal cortex in updating the reward value of internal and external stimuli according to the current homeostatic needs, reduced gray matter volume may be involved in the deficient sensing of comfort that has been reported in association with insomnia (Raymann and Van Someren, 2008). Insufficient sensing, integration and updating of hedonic signals may lead to a reduced orbitofrontal output to the caudate nucleus, a major projection area with an important role in dampening cortical arousal (Stoffers et al., 2014).

In accordance with the round-the-clock occurrence of hyperarousal (Riemann et al., 2010), signatures of hyperexcitability and lack of inhibition are not restricted to the sleep period. Transcranial magnetic stimulation (TMS)-induced hyper-excitability was found to be a trait-like daytime characteristic of insomnia (van der Werf et al., 2010); evoked related potentials (ERP) studies revealed signatures of cortical hyper-excitability, during wakefulness (Bastien et al., 2008; Regestein et al., 1993) and sleep-onset (Kertesz and Cote, 2011; Yang and Lo, 2007), and signatures of inhibitory deficits during wakefulness (Hairston et al., 2010; Kertesz and Cote, 2011), sleep onset (Kertesz and Cote, 2011; Yang and Lo, 2007) and sleep (Hairston et al., 2010). Accordingly, for a physiological understanding of insomnia it seems of value to focus on the balance between excitatory and inhibitory processes (Bastien, 2011; Espie, 2002).
Currently, insomnia disorder is defined exclusively by subjective reports (American Psychiatric Association, 2013). A meta-analysis of the sleep macro-structural alterations of insomnia, assessed through polysomnography (PSG)-derived measures, indicates fragmented sleep, reduced slow wave sleep (SWS) and rapid eye movement (REM) sleep (Baglioni et al., 2014). The quantification of the sleep micro-structural alterations of insomnia, assessed through power spectral analysis of the sleep electroencephalography (EEG), revealed signatures of cortical hyperarousal, indexed by elevated high-frequency power (Bader et al., 2013; Feige et al., 2013). Moreover, power spectral analysis of the wake EEG may also reveal mechanisms of insomnia, since it is known to be a sensitive method to observe cortical dynamics reflecting excitatory and inhibitory processes, related to cognition and vigilance states. A few studies have analyzed the spectrum of wake epochs, recorded before the onset and after the offset of sleep, and consistently found enhanced high frequency power (Corsi-Cabrera et al., 2012; Figueredo-Rodríguez et al., 2009; Freedman, 1986).

Other than these wake EEG epochs during recordings in bed, the resting state during wakefulness has received little attention in insomnia research, and the findings have been inconsistent (for an overview, see Table 1). The overall picture of insomnia abnormalities, emerging from the positive findings obtained in these studies, is that of elevated high-frequency spectral power, in the beta-gamma frequency range (Corsi-Cabrera et al., 2012; Freedman, 1986; Wolynczyk-Gmaj and Szelenberger, 2011), and decreased low-frequency spectral power, observed in the 4-8 Hz band (Wolyńczyk-Gmaj and Szelenberger, 2011), at 9 Hz (Freedman, 1986), and in the 12-14 Hz band (Hauri, 1981). Plausible reasons for the poor reproducibility of the findings include small sample sizes, different inclusion criteria and possible heterogeneity of participants within the studies.

In order to solve the inconsistencies and gain a better understanding of the wake resting state brain activity in insomnia disorder, we undertook a study of adequate sample size, with stringent inclusion criteria, in a standardized setting for data-acquisition. We then performed a thorough analysis of the fine-grained spatial and spectral properties of spontaneous oscillations using high-density EEG. Based on the hyperarousal model of insomnia (Riemann et al., 2010), and based on previous findings of daytime abnormalities of brain structure (Altena et al., 2010), function (Stoffers et al., 2014), and excitability (van der Werf et al., 2010) observed in stringently selected homogeneous samples, we expected that the physiological alterations of insomnia disorder are not restricted to the night; spectral signatures of cortical hyperarousal previously reported in the sleep EEG would also be detectable in the wake EEG.

2. Methods

2.1. Participants and questionnaires

Participants were recruited through advertisement and through the Netherlands Sleep Registry (www.sleepregistry.nl)(Benjamins et al., 2013). Participants were screened via telephone, and
subsequently selected through an intake interview. Exclusion criteria at intake were: any neurological, psychiatric or somatic conditions; the use of sleep medications during the previous two months; previous history of sleep apnea, restless legs syndrome, narcolepsy, overt circadian rhythm disorders or chronic sleep deprivation. The inclusion criteria for the Insomnia Disorder (ID) group were in accordance to DSM, Fifth Edition (American Psychiatric Association, 2013). Consistent with recommended research assessment of insomnia (Buysse et al., 2006), we set additional severity criteria, requiring, during the previous 6 months and more than 3 nights per week, (1) a total sleep time of less than 6.5 hours and (2) a sleep onset latency larger than 30 min or wake after sleep onset larger than 30 min. The controls without sleep complaint (CTRL) group included people that indicated to have no sleep difficulties.

One hundred two participants participated in the study, and were preliminarily assigned to a group. An additional exclusion criterion was based on the Insomnia Severity Index (ISI): a score higher than 7 (threshold for subclinical insomnia)(Bastien et al., 2001) was required for the ID group (four participants excluded) and lower than 8 for the CTRL group (three excluded). Participants filled in the Epworth Sleepiness Scale (Johns, 1991)(ESS), a global measure of the propensity to fall asleep during common daytime activities, in order to control for the influence of sleepiness (see Supplementary material). Participants were given a 7 day sleep-diary (Consensus Sleep Diary, Morning administration (Carney et al., 2012)) to assess subjective sleep continuity and habitual sleep schedules. Weekly medians were obtained for: Sleep Onset Latency (SOL), Number of Wake Bouts (NWB), Wake After Sleep Onset (WASO, total time spent awake between the first time asleep and the last awakening), Bed-Time (time when participant went to bed), Lights-Off (time when participant laid in bed and tried to fall asleep), Lights-On (time when participant woke up for the last time in the morning), Get Up Time (GUT – time when participant gets out of bed in the morning), Time In Bed (TIB – time in minutes from Bed-Time to GUT), Total Sleep Time (TST – time spent asleep during TIB).

Fifty-one participants with ID (42 females), aged (range, M ± SD) 21-69, 5.0 ± 13.4 y, and forty-three CTRL (32 females), aged 22-70, 46.1 ± 14.9 y, were finally included in the study. Volunteers were part of a larger study and participated in more assessments than here analyzed and reported, and were paid 200 € for their time and effort to the complete package of assessments. The study was approved by the ethical committee of the VU University Medical Center, Amsterdam, The Netherlands.

2.2. Electroencephalography recordings
Participants were instructed to maintain a regular sleep/wake schedule during two weeks prior to laboratory assessment. Moreover, on the day of laboratory assessment, they were also instructed to refrain from alcohol and drugs and to limit their intake of caffeinated beverages to a maximum of two cups, which were allowed only before 12:00. The time of onset of EEG recordings (RecT) was scheduled between 19:15 and 23:45 pm, according to the convenience of the participant. Participants were seated in an upright
position and instructed not to move their head and not to fall asleep, in two wake resting-state conditions: 5 minutes of visual fixation on a crosshair on a monitor (eyes open, EO), followed by 5 minutes with eyes closed (EC). High-density EEG (HD-EEG) was recorded using a 256-electrode system, connected to a Net Amps 300 amplifier (Electrical Geodesic Inc., Eugene, OR, input impedance: 200 MΩ, A/D converter: 24 bits). Electrode impedance was kept below 100 kΩ. Signals were acquired with a sampling rate of 1000 Hz and with a Cz reference.

2.3. EEG preprocessing

All preprocessing steps were coded in MATLAB (The Mathworks Inc., Natick, MA; version 8.3), using the MEEGPIPE toolbox (https://github.com/meegpipe/meegpipe). The signals were first filtered in the time domain with a 1D Local Polynomial Approximation filter with adaptive-scale selection from the Local Approximations in Signal and Image Processing (LASIP) toolbox (Katkovnik et al., 2006), to attenuate large non-physiological amplitude components (e.g. sudden spikes and slow fluctuations). Signals were subsequently downsampled to 250 Hz with an antialiasing filter, and then band-pass filtered using a windowed sinc Type I FIR filter (Widmann and Schröger, 2012), with cutoffs at 0.75 and 65 Hz, attenuation of -80dB, transition bandwidth of 0.2 and 5 Hz from each cutoff, respectively. Electrodes, first, and epochs, later, were evaluated for rejection using two similar automated procedures, adaptive for each EEG recording (Supplemental Material). Further artifacts from physiological (heartbeat, eye movements/blinks, muscle tension) and non-physiological (power-line and sparse-sensor noise) sources were removed using automated procedures (Supplemental Material). Importantly with respect to high-frequency EEG investigations, we specifically compensated for signal originating from muscle activity using canonical correlation analysis (De Clercq et al., 2006). Electrodes placed on the neck and face were excluded from further analysis; the remaining 183 scalp electrodes were re-referenced to the common average. We considered it important to evaluate where maximal group differences can be expected in recordings made with a limited number of American Academy of Sleep Medicine (AASM)-recommended electrodes, which are re-referenced to the contralateral mastoid. In order to do so, we conducted a separate set of analyses after re-referencing each electrode to the contralateral mastoid (Supplementary Material) (American Academy of Sleep Medicine, 2007).

2.4. Relative spectral power

For the preprocessed signals of each electrode, spectral power was estimated with a multitaper method (4 orthogonal Slepian sequences, frequency smoothing of 0.8 Hz) implemented in the FieldTrip toolbox (Oostenveld et al., 2011), over 3-sec epochs with 50% overlap, then averaged across epochs. The relative spectral power was obtained by dividing the power in each of the 116 frequency bins in the 1.5-40 Hz range (resolution 0.332 Hz) by the average over this range. This had two purposes: enhancing frequency specific
topographical differences and reducing the influence of sources of variability in broad-band power that were of no interest. Between-electrode variability sources of no interest involve different impedances, contact and gain. Between-subject variability sources of no interest involve differences in anatomy, skin conductance and applications of the net. The resulting electrode by frequency bin matrix was used to investigate fine-grained spatiotemporal group differences.

2.5. Overall low vs high frequency power ratio

The most consistent EEG feature previously reported in ID is high power in the beta-gamma frequency range (Perlis et al., 2001), which has been interpreted as elevated cortical arousal (Bonnet and Arand, 2010a; Freedman, 1986; Riemann et al., 2010; Wolynczyk-Gmaj and Szelenberger, 2011). In addition, some studies during wakefulness found low power across the lower part of the spectrum in the wake EEG: in the 4-8 Hz band (Wolynczyk-Gmaj and Szelenberger, 2011), at 9 Hz (Freedman, 1986), and in the 12-14 Hz band (Hauri, 1981). To obtain a single measure of these opposite lower and higher frequency deviations, we computed the ratio between the lower (LO, 1.5-16 Hz) and upper (HI, 16-40 Hz) part of the spectrum. The 16 Hz cutoff frequency was chosen at the lower end of the beta band, as defined by Klimesch (Klimesch, 2012). The LO/HI RATIO measure was calculated both for each electrode and as scalp-average.

2.6. Statistical analysis: permutation based-inference with Threshold-Free Cluster-Enhancement

Statistical analyses, evaluating group differences on EEG measures, were performed separately for the EO and the EC resting states, with Wilcoxon rank sum tests (Conover, 1980). No transformation was applied to the data when non-parametric statistics were used, since monotonic transformations would have no effect on the rank of the values. For plotting purposes, however, we applied a log10-transformation. Differences between ID and CTRL were first evaluated for the scalp-averaged outcome measures, followed by separate tests for each electrode. Subsequently, the relative power at the more fine-grained spatiotemporal level was investigated separately for the lower and upper parts of the spectrum (1.5-16 Hz and 16-40 Hz respectively). Monte Carlo permutations of group membership labels were used to construct the empirical null hypothesis distribution that the two groups do not differ at any particular spatial or spatiotemporal bin, respectively at the electrode and electrode by frequency level. Threshold-Free Cluster-Enhancement (TFCE) (Mensen and Khatami, 2013) was used to correct for multiple comparisons while evaluating group differences in relative power: (1) at the electrode level, for the LO/HI RATIO; (2) at the electrode by frequency level. TFCE is a non-linear transformation applied to a set of test statistics that takes into account the intensity in the neighborhood of each value. Compared to the more commonly used cluster-based permutations (Maris, 2012), it provides better sensitivity over a wider range of signal types, equally enhancing large narrow effects as well as small broad effects (Mensen and Khatami, 2013). Furthermore, it does not require an arbitrary threshold to define clusters, and it provides an integrated score with its
relative P-value, across all possible thresholds, for each spatial or spatiospectral bin, while compensating for multiple comparisons. In each run of the Monte Carlo permutations, the maximum absolute value is taken after enhancement of the statistic ($Z_{tfce}$), to form the null hypothesis distribution. The $Z_{tfce}$ of the original correct group membership labels is then compared to the null hypothesis distribution, providing the two-sided probability value (P) at each individual spatial and spatiospectral bin.

2.7. Correlation Between Physiological Features of Insomnia
We computed the correlation between the most prominent features of ID that were observed in the exploratory analysis, namely upper alpha in EO and broad beta in EC. To do so, we took a measure for each participant, in EO and EC, respectively the “mean Upper Alpha” and “mean Broad Beta”, by averaging the log 10 of the relative power of all spatiospectral bins that showed a significant effect of ID (Upper Alpha: 81 bins in the 11-12.7 Hz, involving 26 electrodes; Broad Beta: 1275 spatiospectral bins from 16.3 to 40 Hz, involving 124 electrodes). We tested the Spearman correlation between “mean Upper Alpha” and “mean Beta/Gamma”, across all participants, and separately for each group.

2.8. Source reconstruction and group differences at selected frequency bins
To estimate the cortical sources of the relative power at the two frequency bands where we found the largest group differences, in EO and EC, we employed the linearly constrained minimum variance (LCMV) beamforming method (Van Veen et al., 1997), as implemented in Fieldtrip (http://www.fieldtriptoolbox.org/). We used a sparse grid of 90 regions comprising the centroids of all cortical and subcortical areas in the automated anatomical labeling (AAL) parcellation (Tzourio-Mazoyer et al., 2002). A regularization parameter of $\lambda=0.15$ was used for beamforming. Prior to the computation of the covariance matrix, EEG signals were filtered separately using a broadband filter (1-40 Hz) and two narrowband filters: upper alpha (11-13 Hz) and beta (16.3-21.7 Hz). Afterwards, the broadband power at each source location was used to normalize the source power within each narrow band. Finally, the relative source power at each AAL location was compared between controls and patients with insomnia disorder using a two-tailed non-parametric Wilcoxon test. Significant changes in source power are reported at P<0.05 using Benjamini & Hochberg's False Discovery Rate (FDR) procedure to control for multiple comparisons (Benjamini and Daniel, 2001).

3. Results
3.1. Participants, questionnaires and electroencephalography recordings
After artifact rejection (Supplemental Material), two recordings from a participant were excluded due to insufficient length (less than 3 min). The following group statistics are reported in Table 2. Concerning the descriptives, ID and CTRL did not differ significantly in age, nor in sex. Concerning the questionnaires, each
participant with ID had, as expected, larger ISI scores than any of the CTRL; ID and CTRL did not differ with respect to the ESS scores. Sleep diaries revealed that ID—as compared to CTRL—reported significantly longer SOL and WASO, shorter TST lower SE, earlier lights-on time and shorter TIB, but did not differ with respect to Bed-time, Lights-Off and out-of bed time (GUT). The groups also did not differ with respect to the time of the EEG recording.

3.2. Overall low vs high frequency power ratio
People with ID, as compared to controls, had a lower scalp-average LO/HI RATIO both during EO (Z = -1.90, p = .06) and during EC (Z = -2.40, p = .02), resulting either from less low frequency power, or more high frequency power, or both. Subsequent multiple comparison-corrected electrode-wise evaluation of topographical specificity of these group differences (Figure 1) showed a significantly (p < .05) lower LO/HI RATIO in ID during EO in 19 electrodes over midline central regions (peak: Z = -2.84, Ztce = -77.94, p = .04). During EC, people with ID had significantly lower LO/HI RATIO in 85 electrodes distributed over frontal, central and bilateral posterior regions (peak: Z = -3.16, Ztce = -111.21, p = .03).

3.3. Fine-grained spatiotemporal power differences
Figure 2 illustrates that during EO people with ID have less power than CTRL in two narrow upper alpha band spatiotemporal clusters, spanning from 11 to 12.7 Hz, both with maximal differences at 11.7 Hz. One cluster (69 spatiotemporal bins with p < .05, peak Z = -4.05, Ztce = -836.78, p = .03) involved 22 electrodes over left temporal, parietal and frontal regions; the other (12 spatiotemporal bins with p < .05, peak Z = -3.73, Ztce = -773.76, p = .05) involved 4 electrodes over the right frontal region.

During EC, people with ID have more power than CTRL in a large high frequency spatiotemporal cluster spanning from 16.3 to 40 Hz and with maximal differences at 19 Hz. The cluster (1275 spatiotemporal bins with p < .05, peak Z = 3.35, Ztce = 1541.50, p = .003) involved 124 electrodes widely distributed across the scalp: at 19 Hz, over prefrontal, right fronto-temporal, central, and bilateral posterior regions; at higher frequencies, from 22 to 25 Hz, over bilateral parietal, prefrontal and frontal regions; and at even higher frequencies, from 25 to 40 Hz, the cluster involved left parietal, prefrontal and frontal regions.

3.4. Correlation Between Physiological Features of Insomnia
The correlation between the two main features of ID, “mean Upper Alpha” and “mean Broad Beta”, was negative across all participants (ρ = -.37, Z = -3.84, p = .0002), as depicted in Figure 3. In ID this relationship was more marked (ρ = -.32, Z = -2.4, p = .02) than in CTRL (ρ = -.25, Z = -1.7, p = .09).
3.5. **Source reconstruction and group differences at selected frequency bins**

We reconstructed the sources for the frequency bands that showed the strongest group differences at the scalp level, as described in the Methods section. The upper panels of Figs. 4 a and b show estimated standardized (Z-scores) relative power at each of the 90 AAL regions for CTRL (left) and ID patients (right), for beta during EC (Figure 4, a) and for upper alpha during EO (Figure 4, b). The strongest sources for beta comprised midline motor areas and the precuneus, and a lateralization toward the left was observed with strong sources along left primary sensory and motor areas. Upper alpha sources comprised occipital, parietal, inferior temporal and midline orbitofrontal regions.

For both frequency bands, differences between ID and CTRL were widespread, consistent with the scalp results (bottom panel of Figure 2). The increase of power in ID, observed in beta in EC, manifested most strongly bilaterally in motor areas (supplementary motor area and paracentral lobule). The reduction of power in ID, observed in upper alpha in EO, involved most AAL regions, and was the largest in motor, occipital, and inferior temporal regions, as well as bilaterally in the amygdala and parahippocampal gyri.

Figure 4, c and Figure 4, d display all AAL regions ranked by their Z-statistic for the difference between ID and CTRL (for a summary of AAL region label abbreviation and location, see Table S1 and Figure S4 of the supplemental material). The ranking shows that the differences between ID and CTRL are rather global, with only small regional differences in significance.

4. **Discussion**

Whereas daytime complaints are a defining characteristic of insomnia disorder (ID), neurophysiological investigations have focused mostly on sleep. Based on the evidence in ID of a characteristic round-the-clock hyperarousal (Riemann et al., 2010) and of daytime alterations (detailed in the introduction) in structure (Altena et al., 2010), function (Stoffers et al., 2014) and excitability (van der Werf et al., 2010), we expected that the spectral signatures of cortical hyperarousal previously reported in the sleep EEG would also be detectable in the wake EEG. HD-EEG assessed during the wake resting state in adequate sample sizes allowed for a sensitive fine-grained analysis of systematic spatiotemporal differences between people with ID and matched controls.

4.1. **Spectral imbalance in ID**

According to the LO/HI RATIO analysis, we found in ID, both in eyes open and in eyes closed, a shift of the spectral energy from the low frequency range (1.5-16 Hz), to the high frequency range (16-40 Hz). The findings are in agreement with previous reports of reduced low frequency power (Freedman, 1986; Hauri, 1981; Wolyńczyk-Gmaj and Szelenberger, 2011), and increased high frequency power (Corsi-Cabrera et al., 2012; Freedman, 1986; Wolyńczyk-Gmaj and Szelenberger, 2011). The shift of spectral energy from low to
high frequencies is consistent with an altered balance of cortical dynamics, due to either lack of inhibition or hyper-excitation. One of several possible circuits involved in rather global modulation of cortical excitability is the proposed attenuated excitatory input of orbitofrontal neurons to the caudate nucleus—a key structure in moderating the cortical excitability (Stoffers et al., 2014). Other subcortical projections may be of equal importance though: the hyperactivation of the wake-promoting orexin system in the lateral hypothalamus, or the hypofunctionality of the sleep-promoting gamma-aminobutyric acid system in the ventrolateral preoptic nucleus (Riemann et al., 2010).

Fine-grained spatiotemporal analysis allowed us to further characterize this overall imbalance. The strength and spread across frequencies of the effect of ID on the relative power is different for the two parts of the spectrum. Differences with controls during EO are in the upper alpha range, peaking at 11.7 Hz, and are narrow-band and large in magnitude; whereas during EC they are in the broad beta range, peaking at 19 Hz, and are broad-band and small in magnitude.

4.2. Widespread spatial effects
Topographical analysis revealed that the reduction of power in ID in the upper alpha range is mostly expressed in bilateral frontal and left temporoparietal regions during EO (Figure 2); whereas the increase of power in ID in the beta and gamma range is extended over prefrontal, frontal, central, and parieto-occipital regions during EC. Previous work suggested that hyperarousal occurs most pronounced in frontal regions (Nofzinger et al., 2006). Although the increase in frontal power in insomnia does not reach significance at the specific 19 Hz frequency bin of maximal group difference plotted in Figure 2, significance is reached in the 22–40 Hz range. Group differences are observed widely across the scalp, albeit with different magnitude. Please note that the topological area where the band-power is maximal does not necessarily coincide with the area where group differences are the largest, because of the variance of the signal or topographical differences between the groups. Group differences may be pronounced at locations with only moderate power, yet less pronounced at locations with the largest power (Figure S3, supplemental material). In spite of the topographies suggestive of spatial specificity (Figure 2), the observed features of insomnia, namely low upper-alpha (11-13 Hz) power and high broad beta (16.3-40 Hz) power, actually occurred over widespread areas. Given the stringent multiple comparison correction that we applied, some regions just crossed significance threshold, whereas others simply did not.

Source estimates confirmed the widespread nature of the group differences, suggesting a global rather than focal origin of scalp profile of low upper alpha power during EO and high beta power during EC, in people with insomnia. This may either indicate that the ID deviations occur across the cortex, or that their locations are highly variable across individuals with ID. An F-test for equality of variances across groups ruled out the second option (supplementary material).
The findings support rather global differences between people with insomnia and controls, in accordance with the proposed global arousal modulatory role of the caudate, and with the diffused alterations resulting from the imbalance between sleep and wake promoting centers within the hypothalamus (Riemann et al., 2010).

4.3. **Clinical relevance**

The findings have practical implications for EEG studies in insomnia. Although wake EEG is much easier to assess than PSG, we here show that it still retains useful spatiotemporal information that characterizes ID. Whereas a fine-grained HD-EEG approach similar to what we applied is necessary to uncover such characteristics, these may now be assessed with a few well-placed electrodes of the extended AASM montage (American Academy of Sleep Medicine, 2007)(Supplementary Material). Analyses described in detail in the supplemental material show that the F4-M1 bipolar electrode pair can best be evaluated for group differences in upper alpha power around 11.7 Hz during EO. The O1-M2 bipolar electrode pair can best be evaluated for group differences in beta power around 19 Hz during EC.

Future studies could investigate whether these signatures could represent trait-like biomarkers of individuals with insomnia or at risk for insomnia, due to family history, or whether they represent state-like signatures that remits after successful intervention. It could moreover be of interest to investigate whether individual differences in these features predict the response to intervention.

Although the findings reported have practical implications for diagnostic and clinical purposes, these are also highly relevant for the comprehension of the physiopathology on insomnia. In the following paragraphs, we discuss possible functional correlates of enhanced broad beta and attenuated upper alpha band power, and report converging evidence that ID is characterized by hyperarousal and lack of inhibition of cortical dynamics, which in turn may interfere with cognitive and sensorimotor flexibility.

4.4. **Widespread enhanced broad beta band power and hyperarousal**

According to the hyperarousal model of insomnia (Perlis et al., 2001), high power in the broad beta band reflects elevated levels of arousal of the central nervous system and closely matches the hyper-vigilant cognitive style of insomnia disorder. Cortical arousal is a long-standing interpretation for high frequency EEG activity in insomnia. In an early study, Freedman (1986) found high beta power in sleep-onset insomnia during Rapid Eye Movements (REM) sleep and non-REM stage 1 sleep. Recently, a study found high beta power in primary insomnia during resting-state wakefulness (Corsi-Cabrera et al., 2012) in agreement with our observations. However, when considering the same frequency range (17-30 Hz), we did not only observe the reported frontal effect, but a more widespread effect, that encompassed prefrontal, frontal, central, right temporal and bilateral posterior regions on the scalp, pointing to global hyperarousal. Source analysis, at the beta frequency bin where we observed maximal evidence of group differences (19 Hz),
confirmed that source-level differences were also widespread. The differences were maximal over sensorimotor cortices, the region were the sources mostly originated from. Furthermore the differences extended over prefrontal, frontal, right temporal regions of the cortex. These results are suggestive of a global hyperarousal, encompassing sensorimotor, cognitive, and emotion-regulation systems. In healthy awake volunteers, EEG activity in the beta range is implicated in cognition, attention, and perception (Lopes da Silva, 1991). However, pathologically increased beta power is thought to interfere with cognitive and behavioral flexibility, by rigidly maintaining the cognitive and sensorimotor status quo (Engel and Fries, 2010). Follow-up studies have to investigate whether the enhanced beta we observed in the awake EEG of people suffering from ID could be involved in their reportedly hampered ability to disengage from cognitive, sensorimotor and emotional processes (Bastien, 2011).

Although the maximal differences we observed are near the beta peak frequency, our findings extend into the gamma range up until the last frequency bin we studied (16.3–40 Hz). In healthy awake volunteers, gamma band activity in the range of 30–100 Hz is implicated in learning, focused attention, memory and sensory processing (Kaiser and Lutzenberger, 2005). In one EEG-functional magnetic resonance imaging (fMRI) study (Chen et al., 2014), females suffering from ID showed high gamma power during perisleep epochs. Gamma power fluctuations were moreover associated with fluctuations in the blood oxygenated level dependent (BOLD) signal in the insula, suggesting pathologically elevated somatosensory awareness and distress. It is tempting to suggest that enhanced beta and gamma power could be involved in cognitive complaints characteristic of ID, including ruminations, worries, racing thoughts and enhanced interoception, exteroception, and self-referential processing.

4.5. **Upper alpha band role in the inhibition of interfering cognitions**

The functional relevance of attenuated upper alpha power relies on the association of alpha with inhibition. Large amplitude alpha oscillations have been suggested to reflect a state of cortical deactivation or active inhibition (Hummel et al., 2002). Consistently, in some studies, alpha amplitude is inversely related to cortical excitability (Klimesch, 1999, 2012). In combined EEG-fMRI studies, the BOLD signal in widespread cortical regions is anti-correlated with the estimated localized cortical alpha amplitude (Goldman et al., 2002). Strong negative BOLD-alpha power correlations are not limited to the occipital cortex, but are also found at cortical sites further away from the generators in the occipital lobe (Gonçalves et al., 2006; Laufs et al., 2003). Furthermore, transient periods of high alpha activity are paralleled by a decrease in BOLD connectivity, consistently with the idea that alpha may represent inhibition across a distributed network of cortical areas (Chang et al., 2013; Scheeringa et al., 2012; Tagliazucchi et al., 2012). Whereas source analysis indicated that low power in the upper alpha band in insomnia occurred globally, the difference with controls was most significant in visual, somatosensory, and motor areas. Given the role of alpha oscillations in
inhibition, this finding suggests a global insufficiency in inhibition that may show most strongly for sensory and motor processing.

The theoretical framework put forward by Klimesch links specifically the cortical inhibitory role of the upper portion of the alpha range, to the selection and suppression of irrelevant and interfering cognitions (Klimesch, 2012; Klimesch et al., 2007). In his words, referring to the inhibitory function of alpha: “large resting power may reflect a person’s ability to build up a highly efficient filter” (Klimesch, 2012). Widespread lack of cortical inhibition, paralleled by reduced selectivity of the attentional filter, could therefore give space to ruminations and interfering cognitions.

In an early neurofeedback experiment, participants with obsessive rumination were trained to modulate alpha power, and indeed an inverse association of alpha power with rumination was found (Mills and Solyom, 1974). Given the importance of rumination in insomnia, it would be interesting to investigate whether reduced upper alpha power mediates the tendency to ruminate among people suffering from insomnia, and whether neurofeedback targeting this frequency range could thus alleviate cognitive symptoms in insomnia. Recently, a neurofeedback training successfully enhanced relative power, specifically in the upper alpha band, yielding benefits in attention and impulsivity, that are consistent with increased efficiency of the attentional inhibitory filter (Escolano et al., 2014). It may be possible that the success of neurofeedback training in ID,(Cortoos et al., 2010; Hauri, 1981; Hauri et al., 1982; Hoedlmoser et al., 2008; Schabus et al., 2014) targeting the sensorimotor rhythm in the 12–15 Hz range, was partially due to the overlap between this band and the upper alpha band (11–13 Hz), the band where we observed the most impairment in ID. Future neurofeedback studies in insomnia should specifically target the upper-alpha oscillations, where we observed the largest physiological alterations of ID.

In sum, it is tempting to suggest a possible role of attenuated alpha power in the inability to suppress intrusive cognitions (Bazanova and Vernon, 2014) and the resulting ruminations that exert a pivotal role in the etiology of ID (Riemann et al., 2010).

4.6. Control of possible confounders
It is unlikely that our finding of enhanced broad beta power is of electromyographic origin. First, we specifically addressed signals originating from muscle activity using canonical correlation analysis (De Clercq et al., 2006). Second, the group-difference effect size as reflected in the number of significant electrodes tended to decrease with frequency, rather than increase as it would be expected if the effect was driven by muscular activity under common average reference (Goncharova et al., 2003). Sleepiness is known to affect the EEG spectral power, in complex ways (Ogilvie, 2001). The amplitude of spontaneous oscillations in the broad alpha band during eyes open has been shown to be positively related to sleep pressure and fatigue (Åkerstedt and Gillberg, 1990; Cajochen et al., 1995; Kaida et al., 2006; Stampi et al., 1995), whereas the inverse holds during eyes closed resting state (Åkerstedt and Gillberg, 1990;
Curcio et al., 2001; Kaida et al., 2006; Stampi et al., 1995) during wakefulness or the transition to sleep (Klimesch, 1999; Ogilvie, 2001). We obtained a measure of state-sleepiness by quantifying the attenuation of the alpha power, in EC relative to EO, by means of the Alpha Attenuation Index (AAI)(Putilov and Donskaya, 2014; Stampi et al., 1995)(Supplementary Material). We moreover assessed the ESS (Johns, 1991) as a measure of habitual sleepiness. We found no differences between ID and CTRL in either AAI or ESS. It is therefore highly unlikely that our findings involve group differences in either habitual or momentary sleepiness.

Resting state alpha power starts to decrease considerably after the age of about 50 years (Breslau et al., 1989), especially in the upper alpha band. In the current study, the two groups did not differ significantly in age (mean difference = 3.97 y, \( p = .22 \)). We then controlled for the possible confounding effect of age, by means of analysis of covariance (Supplementary Material). The main effect of ID on its electrophysiological correlates remained significant after taking into account the variation entailed by age; furthermore, the interaction of age with ID did not significantly affect the electrophysiological correlates of ID.

Finally, benzodiazepines are known to suppress alpha and increase sigma and beta amplitude (Feshchenko et al., 1997). In the present study, use of benzodiazepines was an exclusion criterion.

In summary, we excluded that the groups were different with respect to possible confounders (habitual and momentary sleepiness, age, sex, time of the recording, typical bedtime and typical GUT).

5. Conclusions

Our findings on the fine-grained spatiotemporal characteristics of the wake resting-state HD-EEG in people suffering from insomnia disorder points to an imbalance of cortical excitation and inhibition, as indexed by a shift in spectral energy from low to high frequencies. During eyes open, people suffering from insomnia show lower power than controls in the upper alpha range, with maximal evidence at 11.7 Hz. This difference with controls is significant only in a narrow band. During eyes closed, people suffering from insomnia show higher power in the broad beta range, with maximal evidence at 19 Hz. This difference with controls extends over a rather broad frequency range. There is no evidence for a localized effect in either condition, as assessed by source-analysis. Furthermore, we provide recommendations on where to best observe these differences on the scalp, using bipolar electrodes of the AASM extended montage. Functional interpretation of the spectral alterations points to hyperarousal and lack of inhibition. The broad-band small increase in the beta range supports hyperarousal of the central nervous system; the narrow-band large reduction in the upper alpha range possibly reflects reduced cortical inhibition and reduced selectivity of the attentional filter.
6. Figures

Figure 1—Imbalance between low and high frequency spectral power in insomnia, during the eyes open (EO) and eyes closed (EC) resting state. As compared to controls without sleep complaints (CTRL), individuals with insomnia disorder (ID) show a low ratio between the power of the lower (1.5–16 Hz) over upper (16–40 Hz) part of the spectrum (LO/HI RATIO). This was most pronounced over midline anterior (prefrontal, frontal, and central) and left parietal regions during EO, and over midline anterior (prefrontal, frontal, central) and bilateral posterior regions (temporal, parietal) during EC. The three maps on the left side show the results for EO, the three maps on the right side the results for EC. Of these triplet maps, the first and the second show the topographical distribution of the within-group median LO/HI RATIO, whereas the third map shows the relative group difference statistic (Z). Electrodes where the p value (derived from the Threshold-Free Cluster-Enhancement procedure) is less than 0.05 are marked with a black dot.
Figure 2—Less upper alpha and more broad beta relative power in insomnia, respectively, during the eyes open (EO) and eyes closed (EC) resting state. Compared to controls without sleep complaints (CTRL), individuals with insomnia disorder (ID) have less power in a narrow upper alpha frequency band over a bilateral frontal and left temporoparietal region during EO. During EC they show more power in a broad beta band over widespread regions. The left and right side of the figure show the results for the EO and EC resting state, respectively. The top row shows the log-transformed relative spectral power, averaged across scalp electrodes, of the two groups (red for ID, blue for CTRL). For each frequency bin and for each of the two groups separately, the median value (line) and its 95% bootstrap confidence interval (semitransparent area) across participants is shown. The middle panels show the percentage of electrodes that are significantly different between groups at each frequency bin, when the p values (derived from the Threshold-Free Cluster-Enhancement procedure) are thresholded at 0.1 (grey) or 0.05 (black). The bottom panels show the topographical distribution of the within-group median log-transformed spectral power and the relative group difference statistic (Z), at the frequency bin where the largest number of significant electrodes was found, respectively 11.7 Hz in the EO and 19 Hz in the EC condition (indicated by the dashed line in the top panel). Electrodes where p is less than .05 are marked with a black dot.
Figure 3— Mean upper alpha in eyes open (EO) is moderately anticorrelated with mean broad beta in eyes closed (EC), in both controls (CTRL, blue dots) and, more pronouncedly, in insomnia (ID, red dots). Least square lines are fit within each group (red and blue lines for ID and CTRL respectively), and across groups (black dashed line); ρ and probability-value (P) of the respective Spearman correlations are shown on top. The large unexplained variation suggests that expressing low upper alpha power in EO does not necessarily entail expressing high broad beta power in EC. Upper alpha and broad beta likely express two distinct, interrelated, functional processes.
Figure 4—Source localization reveals a widespread alteration of oscillatory power in insomnia disorder: decreased upper alpha band power during eyes open (EO) and increased beta band power during eyes closed (EC). 

a) Upper panel: distribution of beta sources obtained using linearly constrained minimum variance beamforming, for insomnia disorder (ID, left) and controls without sleep complaints (CTRL, right). Average source power was converted to z-scores for visualization. Bottom panel: Automatic anatomical labelling (AAL) template regions where significant ($p < .05$, Wilcoxon test, Benjamini-Hochberg false discovery rate correction) increases in beta oscillatory power in ID vs. CTRL were observed. 

b) Upper panel: distribution of upper alpha sources for ID (left) and CTRL (right)—z-scored for visualization. Bottom panel: AAL template regions where significant decreases in upper alpha oscillatory power ID versus CTRL were observed. 

c) AAL regions ranked according to the effect size of the beta increases in ID versus CTRL (for more information on AAL regions, including abbreviations, please see Figure 4 and Table 1 of the Supplementary Information). 

d) AAL regions ranked according to the effect size of the upper alpha decreases in ID versus CTRL.
7. Tables

Table 1-Summary of findings in insomnia based on power spectral analysis of the wake EEG

<table>
<thead>
<tr>
<th>Study</th>
<th>Participants</th>
<th>Setting</th>
<th>Findings (case vs control)</th>
<th>Electrodes</th>
<th>Power spectral analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wu et al., 2013</td>
<td>50 PI, 32 controls</td>
<td>Evening EC</td>
<td>NS</td>
<td>C3, C4, Ref: A1+A2</td>
<td>Abs 1 Hz bin, .5-32 Hz range</td>
</tr>
<tr>
<td>Corsi-Cabrera et al., 2012</td>
<td>10 young PI, 10 controls</td>
<td>Wake epochs during the wake to sleep transition period</td>
<td>More frontal beta power, more frontoparietal coupling within beta and gamma</td>
<td>19 electrodes, 10-20 system, Ref: average.</td>
<td>Abs, beta (17-30 Hz), gamma (31-45 Hz).</td>
</tr>
<tr>
<td>Wolynczyk-Gmaj and Szelenberger 2011</td>
<td>36 PI, 29 controls</td>
<td>First 2 minutes of 4 sessions from the multiple sleep latency test</td>
<td>Less theta (4-8 Hz), more beta (18-30 Hz)</td>
<td>Average of Fp1, Fp2, Fpz, Ref: bilateral between Fz and Cz</td>
<td>Rel, 7 bands (delta, theta, alpha, beta1, beta2, beta3), 1-30 Hz range</td>
</tr>
<tr>
<td>Bucklew et al. 2009</td>
<td>20 students, poor vs good sleep (N = NR)</td>
<td>EO, EC, audio-listening, mental operation</td>
<td>Sustained theta, not suppressed from EC to task</td>
<td>C3, Ref: bilateral earlobes</td>
<td>Abs theta (4-8 Hz), beta (13-21 Hz)</td>
</tr>
<tr>
<td>Freedman 1986</td>
<td>12 sleep-onset PI, 12 controls</td>
<td>EO seated, EC in bed</td>
<td>Less alpha (9 Hz), more Abs. beta occipital</td>
<td>C3, O1, Ref: A2</td>
<td>Abs, 1 Hz bin, .5-30 Hz range</td>
</tr>
<tr>
<td>Hauri 1981</td>
<td>10 with self-reported insomnia, 10 controls</td>
<td>2 wake noontime sessions</td>
<td>Less SMR</td>
<td>C3, Ref: A2</td>
<td>Abs SMR (12-14 Hz)</td>
</tr>
</tbody>
</table>

PI, Primary Insomnia; Abs, Absolute band power; Rel, band power relative to total power; Ref, reference; NS not significant; NR not reported; SMR, sensorimotor rhythm; EO, eyes open; EC, eyes closed.
Table 2- Demographic variables, questionnaires, recording time and sleep diary variables, in insomnia disorder (ID) and controls without sleep complaints (CTRL)

<table>
<thead>
<tr>
<th></th>
<th>ID (N=53) range, M ± SD</th>
<th>CTRL (N=43) range, M ± SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>9/42</td>
<td>11/32</td>
<td>NS</td>
</tr>
<tr>
<td>Age, y</td>
<td>21-69, 50.0 ± 13.4</td>
<td>22-70, 46.1 ± 14.9</td>
<td>NS</td>
</tr>
<tr>
<td>ISI</td>
<td>8-25, 16.0 ± 4.3</td>
<td>0-7, 1.9 ± 1.9</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>ID duration, y</td>
<td>.5-54, 21.98 ± 15.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESS</td>
<td>0-19, 6.3 ± 4.43</td>
<td>1-14, 6.74 ± 3.21</td>
<td>NS</td>
</tr>
<tr>
<td>RecT</td>
<td>20:34-23:37, 22:09 ± 0:39</td>
<td>20:26-23:43, 22:05 ± 0:54</td>
<td>NS</td>
</tr>
<tr>
<td>SOL, min</td>
<td>1-180, 31.5 ± 36.35</td>
<td>0-30, 11.4 ± 7.89</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>NWB</td>
<td>0-8, 2.59 ± 1.66</td>
<td>0-4, 1.60 ± 1.16</td>
<td>.002</td>
</tr>
<tr>
<td>WASO, min</td>
<td>3-180, 60.12 ± 47.34</td>
<td>0-90, 15.12 ± 15.96</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>TST, min</td>
<td>223.0-495.5, 380.9 ± 59.2</td>
<td>3824-580.0, 454.1 ± 39.1</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Bed-Time</td>
<td>21:10-1:25, 23:12 ± 0:50</td>
<td>21:00-2:00, 23:27 ± 0:59</td>
<td>NS</td>
</tr>
<tr>
<td>Lights-Off</td>
<td>22:15-1:30, 23:36 ± 0:40</td>
<td>22:01-03:00, 23:50 ± 0:52</td>
<td>NS</td>
</tr>
<tr>
<td>Lights-On</td>
<td>3:00-9:17, 6:59 ± 1:08</td>
<td>4:00-11:15, 7:23 ± 1:04</td>
<td>NS</td>
</tr>
<tr>
<td>GUT</td>
<td>6:00-11:15, 7:55 ± 1:07</td>
<td>5:55-11:30, 7:51 ± 0:57</td>
<td>NS</td>
</tr>
<tr>
<td>TIB, min</td>
<td>386.5-705, 495.7 ± 61.5</td>
<td>360-580, 485.5 ± 46.7</td>
<td>NS</td>
</tr>
</tbody>
</table>
8. Acknowledgments
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9. References


