Alpha-wave frequency characteristics in health and insomnia during sleep

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SUMMARY

Appearances of alpha waves in the sleep electrencephalogram indicate physiological, brief states of awakening that lie in between wakefulness and sleep. These microstates may also cause the loss in sleep quality experienced by individuals suffering from insomnia. To distinguish such pathological awakenings from physiological ones, differences in alpha-wave characteristics between transient awakening and wakefulness observed before the onset of sleep were studied. In polysomnographic datasets of sleep-healthy participants ($n = 18$) and patients with insomnia ($n = 10$), alpha waves were extracted from the relaxed, wake state before sleep onset, wake after sleep-onset periods and arousals of sleep. In these, alpha frequency and variability were determined as the median and standard deviation of inverse peak-to-peak intervals. Before sleep onset, patients with insomnia showed a decreased alpha variability compared with healthy participants ($P < 0.05$). After sleep onset, both groups showed patterns of decreased alpha frequency that was lower for wake after sleep-onset periods of shorter duration. For patients with insomnia, alpha variability increased for short wake after sleep-onset periods. Major differences between the two groups were encountered during arousal. In particular, the alpha frequency in patients with insomnia rebounded to wake levels, while the frequency in healthy participants remained at the reduced level of short wake after sleep-onset periods. Reductions in alpha frequency during wake after sleep-onset periods may be related to the microstate between sleep and wakefulness that was described for such brief awakenings. Reduced alpha variability before sleep may indicate a dysfunction of the alpha generation mechanism in insomnia. Alpha characteristics may also prove valuable in the study of other sleep and attention disorders.

INTRODUCTION

The frequency of alpha waves visible in the electrencephalogram (EEG) is a remarkably stable trait in healthy humans with a population standard deviation of only about 1 Hz (Grandy *et al.*, 2013; Niedermeyer, 1997). Larger deviations from this physiological alpha range have been associated with pathological processes, such as deteriorating working memory performance (Klimesch *et al.*, 1993; Richard Clark *et al.*, 2004), negative symptoms in schizophrenia (Garakh *et al.*, 2011), Alzheimer's disease (Moretti *et al.*, 2007; Ponomareva *et al.*, 2013), or addiction (Domino *et al.*, 2009). Alpha frequency, amplitude and phase also show physiological correlations with cognitive performance, visual attention, memory and complex, abstract tasks (Angelakis *et al.*, 2004; Busch and VanRullen, 2010; Hanslmayr *et al.*, 2011; Klimesch, 1999).

The correlation of EEG alpha waves and attentional states is particularly pronounced in the process of falling asleep, where alpha decay is a major hallmark (Ogilvie, 2001). A desynchronization or a destabilization of the alpha-rhythm-generating process may underlie this disappearance of EEG alpha. In either case, one would expect the rhythm to become increasingly imprecise before its amplitude decays. Some

evidence of a relation between such alpha frequency fluctuations and attention was given by Jin *et al.* (2006). The authors describe correlations between reaction times of a visual attention task and the width of the spectral alpha peak measured at later times.

After sleep onset, alpha waves are found during brief episodes of wakefulness interrupting sleep (wake after sleep-onset periods, WASOs) and during arousals of sleep (Halász *et al.*, 2004; Schieber *et al.*, 1971; Steriade *et al.*, 1993). Such transient awakenings show characteristic differences from relaxed wakefulness. Depending on the duration of awakenings, individuals may not form memories, and their conscious perception is reduced. Other differences are already expressed in the statistical signature of awakenings, for which Lo *et al.* (2002, 2004) found that awakening durations follow a scale-free distribution. In contrast, durations to sleep onset in multiple sleep latency tests follow distributions with exponential tails (Erkki Kronholm, personal communication, 18 July 2015; Kronholm *et al.*, 1995). Moreover, Cantero *et al.* (1999) found the spectral composition of the alpha band to be state-dependent when comparing states of wake, drowsiness and alpha bursts in rapid eye movement (REM) sleep (for review, see Cantero *et al.*, 2002).

Complaints of daytime dysfunction and disturbing awakenings from sleep in the absence of external stimuli, or respiratory and motoric events signify central insomnia. The subjective reduction in sleep quality likely results from a chronic, pathological state of hyperarousal (Feige *et al.*, 2013; Riemann *et al.*, 2010, 2015). Such hyperarousal is also reflected in increased EEG spectral powers at alpha, sigma and beta frequencies, as well as decreased slow-wave powers during sleep (Freedman, 1986; Merica *et al.*, 1998; Spiegelhalder *et al.*, 2012). However, polysomnographic (PSG) parameters of sleep macrostructure, such as sleep efficiency (SE) or arousal index, often do not confirm the subjective perception of a reduced sleep quality (Baglioni *et al.*, 2014; Edinger *et al.*, 2000; Rosa and Bonnet, 2000). One possible reason is that such metrics do not distinguish between sleep disturbances and physiological arousals known to be part of healthy sleep (Halász *et al.*, 2004). Clearly, physiological and pathological brief awakenings require better characterization. An analysis restricted to arousals from the REM stage differentiated better between healthy subjects and those suffering from insomnia (Feige *et al.*, 2008), leading to the hypothesis of REM sleep instability (Feige *et al.*, 2013). Another refined classification of arousals during sleep has been explored in the study of cyclic alternating patterns (CAPs) of the EEG (for review, see Parrino *et al.*, 2012). In particular, A3-CAPs resembling cortical arousals were found with increased frequency across diverse neurological disorders affecting sleep. These recent findings highlight the possible benefit of untangling the microstructure of sleep (Feige *et al.*, 2013).

To further enhance the characterization of physiological and pathological brief awakenings, this study set out to explore the dynamics of the dominant EEG patterns observable during these microstates of sleep, i.e. alpha waves. It was analysed how alpha frequency changes across these states compared with the wakefulness prior to sleep. Further, the concept of alpha instability and destabilization was explored by investigating to what extent alpha frequency fluctuates on a short time scale.

This analysis focused on properties of alpha frequency and its variability that have been largely disregarded in previous studies (except Jin *et al.*, 2006). Based on the assumption that a rhythm-generation process actively controls the frequency of alpha-wave activity in the brain, three hypotheses were formulated: (H1) the alpha frequency fluctuates around a state-dependent set point; (H2) the set-point frequency reflects the neurophysiological state (Cantero *et al.*, 1999; Nunez *et al.*, 1978); (H3) the frequency variability indicates pathological processes.

MATERIALS AND METHODS

Study participants

The database was searched for PSG records of participants suffering from insomnia [$n = 10$; age: 54 ± 10 years; body mass index (BMI): 25.5 ± 4.0 kg m^{-2}] and participants that were sleep-healthy ($n = 18$; age: 44 ± 8 years; BMI: 26.1 ± 3.0 kg m^{-2}). All participants were male, and age differences between groups were significant ($P = 0.013$, Wilcoxon rank-sum test). Patients with insomnia were neither habituated to nor administering medications that affected their sleep or attention. This study has been approved by the local ethics committee of Charité, Berlin. All participants provided written, informed consent to participate in the study.

Polysomnography had been performed on the second night in the sleep laboratory after 1 night of habituation with about 8 h between lights off and lights on. All participants had been asked to refrain from alcohol, coffee, cigarettes and exuberant nightly activity on the days before the study.

In addition, patients with insomnia received a sleep diary to control regularity of their sleep habits in the days preceding the study. Actigraphy had not been used. All records of standard PSG were scored by three trained and certified sleep scorers primarily using channel combination C3-A1. In accord with the scoring, the same derivation was used in the current investigations. Additional information is available in the original publication of data for healthy participants (Penzel *et al.*, 2012).

Data selection

The sleep record and hypnogram of each participant was scanned for stage W, and these segments were grouped according to three conditions. First, wake before sleep onset and WASOs were regarded separately. Adjacent epochs of WASOs were concatenated, and divided into long and short (duration <5 min) WASOs. Lastly, all EEG recordings were scanned for short arousals (duration <15 s). Subsequent

analyses were performed on each of these modalities (wakefulness, long WASOs, short WASOs and arousals) separately. Note that alpha waves appearing after sleep in the morning and during epochs of REM sleep were disregarded in this study because enough of such segments was not found in the datasets.

For each raw dataset and modality, artefact-free EEG segments were extracted, which were free of eye, muscle, EKG (electrokardiogram), sweat and other artefacts. By manually indicating about 1 min of artefact-free waking EEG for each participant, artefact threshold values for the maximal EEG power in a low-frequency band (1–6 Hz for eye and movement artefacts) and a high-frequency band (20–40 Hz for muscle artefacts) were determined. The remainder of the raw EEG data was then automatically swept for segments with band powers below the artefact thresholds by using a kernel-based support-vector machine. All data were visually examined for EEG artefacts to control this semiautomatic procedure. Specifically, it was checked that no artefacts were present in segments characterized as artefact-free (false negatives), and that data classified as artefacts were indeed in a 3-s vicinity of an artefact (false positives). Furthermore, special care was taken for the state of wakefulness at the beginning of each recording. Only those epochs were considered wherein the participant was in bed with closed eyes to assure similar behavioural resting states for each participant.

Alpha metrics and alpha-to-alpha intervals

As demonstrated in Fig. 1, alpha metrics from alpha-to-alpha intervals (AAIs) were computed, i.e. time intervals between consecutive alpha-wave maxima in the EEG channel C3-A1. First, it was checked whether the peak-to-peak amplitude of each alpha wave exceeded an amplitude threshold (shaded area in Fig. 1a). In such alpha-positive segments, the time points *t_n* of each alpha-wave maximum (vertical lines) were determined. AAIs were computed as the consecutive differences $t_{n+1} - t_n$, whose inverse yielded an estimate of the instantaneous frequency f_n (Fig. 1b). From such sequences, the alpha frequency was computed as the median of f_n and the alpha variability as the f_n – standard deviation, as illustrated in Fig. 1c.

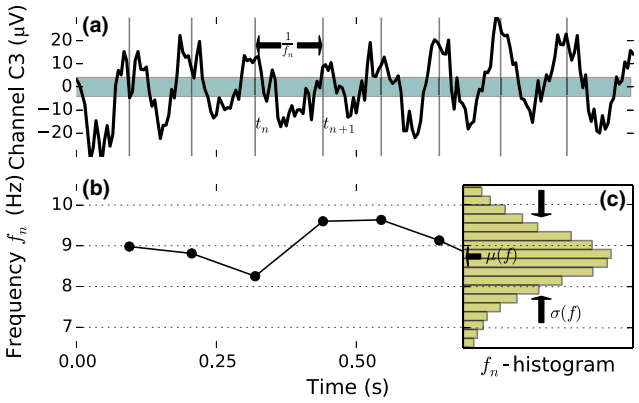

Figure 1. Extraction of alpha wave frequency and variability. (**a**) An artifact-free segment of EEG activity (black line) shows alpha waves, each exceeding a threshold peak-to-peak amplitude of 8 μV (shaded region). We determined the times, t_n, at which the wave form reached their positive peaks (vertical lines), and from these times we determined an instantaneous frequency: $f_n = (t_{n+1} - t_n)^{-1}$. (**b**) The corresponding instantaneous frequency f_n forms a fluctuating sequence. (**c**) We characterize this sequence by its median, $\mu(f)$, indicating alpha frequency, and its standard deviation, $\sigma(f)$, indicating alpha variability, as highlighted in the histogram of all identified f_n in the inset.

Varying numbers of AAIs were found in each modality depending on the segment length, artefact contamination and EEG type of the participant. To facilitate a comparison across participants, therefore a fixed number of 300 AAIs was selected for the wake modality, 100 AAIs for long and short WASOs, and at least 10 AAIs for brief arousals.

The extraction of AAIs was automated in the following steps. Artefact-free segments (see 'Data selection') were band-pass filtered between 7 and 13 Hz using a Butterworth filter with stop band 3.5 and 26 Hz. The filtered signal was Hilbert-transformed and decomposed into the Hilbert phase and radius (Pikovsky *et al.*, 2001). The radius was used as an estimate for half of the peak-to-peak amplitude, and the phase as an estimate of the alpha-wave phase. A fixed threshold was applied to this amplitude (twice the radius); only those segments of the signal whose amplitude variable was larger than the threshold and which were longer than 0.3 s were regarded as alpha-positive. This interval corresponded roughly to three completed alpha waves. This condition aimed to avoid spurious segments of super-threshold amplitude, and to assure that at least one full oscillation was present within an alpha-positive segment, which was always the case. Several amplitude threshold values in their effect on alpha metrics were tested to investigate the dependence on this crucial parameter. In such alpha-positive segments, the phase variable was used to extract AAIs. The time instances were marked in which the Hilbert phase crossed 90 degrees. This phase value corresponds to the alpha-wave maximum. The intervals in between consecutive crossing times, i.e. the AAIs, were further analysed. Their inverse yields an estimate of instantaneous frequency. Thus, alpha frequency and variability were quantified by the median and the standard deviation of these instantaneous frequency sequences, respectively. The conceptual similarity of the AAI analysis to the analysis of beat-to-beat intervals derived from the electrocardiogram can be briefly pointed out.

RESULTS

PSG characteristics

The hypnograms of the sleep-healthy and insomnia groups were evaluated for total sleep time (TST), SE and minutes spent in sleep stages (including stage W). All of these derived

Table 1 *P*-values of alpha-wave metrics comparing different modalities

	W	*S1*	*S2*	*S3*	*S4*	*REM*	*TST*	*SE (%)*
Healthy	61 (33)	27 (11)	140 (37)	69 (22)	76 (33)	93 (25)	404 (60)	87 (8)%
Insomnia	169 (44)	27 (14)	146 (36)	38 (20)	14 (22)	39 (19)	295 (81)	63 (14)%
P-values	<0.0001	<0.0001	0.47	<0.01	<0.0001	<0.0001	<0.001	<0.0001

Metrics of alpha-wave frequency and variability in the wake state (W) are compared with long WASOs (L), short WASOs (S) and arousals from sleep (A). Sleep stages and TST are given in minutes. All values given as mean (standard deviation).

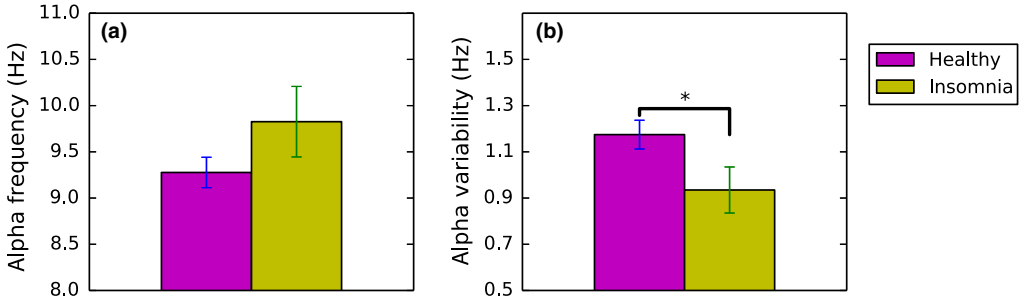

Figure 2. Alpha variability distinguishes the healthy from insomnia group during the wake state. (a) The averaged alpha frequencies of the healthy (n = 14) and insomnia group (n = 8) do not differ significantly. (b) The alpha variability is significantly lower in the insomnia group when comparing with the healthy group (indicated *P*-values, Wilcoxon rank-sum test). Metrics of each individual were computed from the last 300 alpha-to-alpha intervals before sleep onset. Bar heights indicate group means, and error bars indicate their standard error. *P<0.05

measures were compared across the groups using Wilcoxon rank-sum tests. The comparison showed significant ($P < 0.05$) differences for stages W, S1, S3, S4, TST and SE. Healthy participants spent more time in deep sleep (S3 and S4), whereas participants with insomnia spent more time in stages W and S1. Time spent in S2 was balanced. The difference in SE, which was found to be 63 ± 14% for participants with insomnia and 86 ± 8% for healthy participants, was significant; also the TST differed by about 100 min in the mean. Averaged data are summarized in Table 1.

Alpha characteristics during relaxed wakefulness

In the wake state, 300 AAIs of waves exceeding an amplitude threshold of 8 μV were found for 14 healthy participants and nine patients with insomnia. The used threshold value yielded a good trade-off between data quality and the number of individuals with sufficient data as indicated by the results in 'Threshold dependence of alpha metrics'. The AAIs were used to estimate alpha frequency and variability. A group-averaged alpha variability of 1.17 ± 0.06 Hz (mean ± standard error) was estimated for the healthy group, and 0.93 ± 0.1 Hz for the insomnia group. The significance of group differences with $P = 0.042$ was established with a Wilcoxon rank-sum test. Respective alpha frequencies, 9.27 ± 0.16 Hz for healthy participants and 9.83 ± 0.38 Hz for patients with insomnia, were not significantly different from each other ($P = 0.40$). These results are summarized in Fig. 2.

Alpha characteristics during sleep

After sleep onset, alpha waves were found during long and short (duration <5 min) WASOs and during arousals of sleep. For each participant and modality, AAIs were selected (300 for wakefulness, 100 for WASOs and at least 10 for arousals) to estimate the alpha frequency and variability.

Each individual showed a characteristic alpha signature across the modalities, as exemplified in Fig. 3c, where alpha metrics and hypnogram were overlaid for a patient with insomnia. It is particularly apparent in the example that short WASOs differed from long ones in their alpha frequency and variability: the longest WASO starting at 01:00 hours showed a frequency and variability similar to relaxed wakefulness, whereas the series of shorter WASOs around 00:00 hours showed a larger alpha variability and a lower alpha frequency.

Next, alpha metrics of each individual were averaged across events of the same modality, i.e. wake state, long and short WASOs, and arousals. Within each group, the statistical signature of these averaged alpha metrics was investigated by performing pair-wise comparisons between modalities using paired Wilcoxon rank-sum test. All *P*-values are summarized in Table 2.

Compared with wake levels, the insomnia group showed a reduction in alpha frequency for WASOs (short WASOs: $P = 0.008$; long WASOs: $P = 0.016$). The reduction was graded in that short WASOs also showed a significantly lower frequency than long ones ($P = 0.02$). Alpha variability

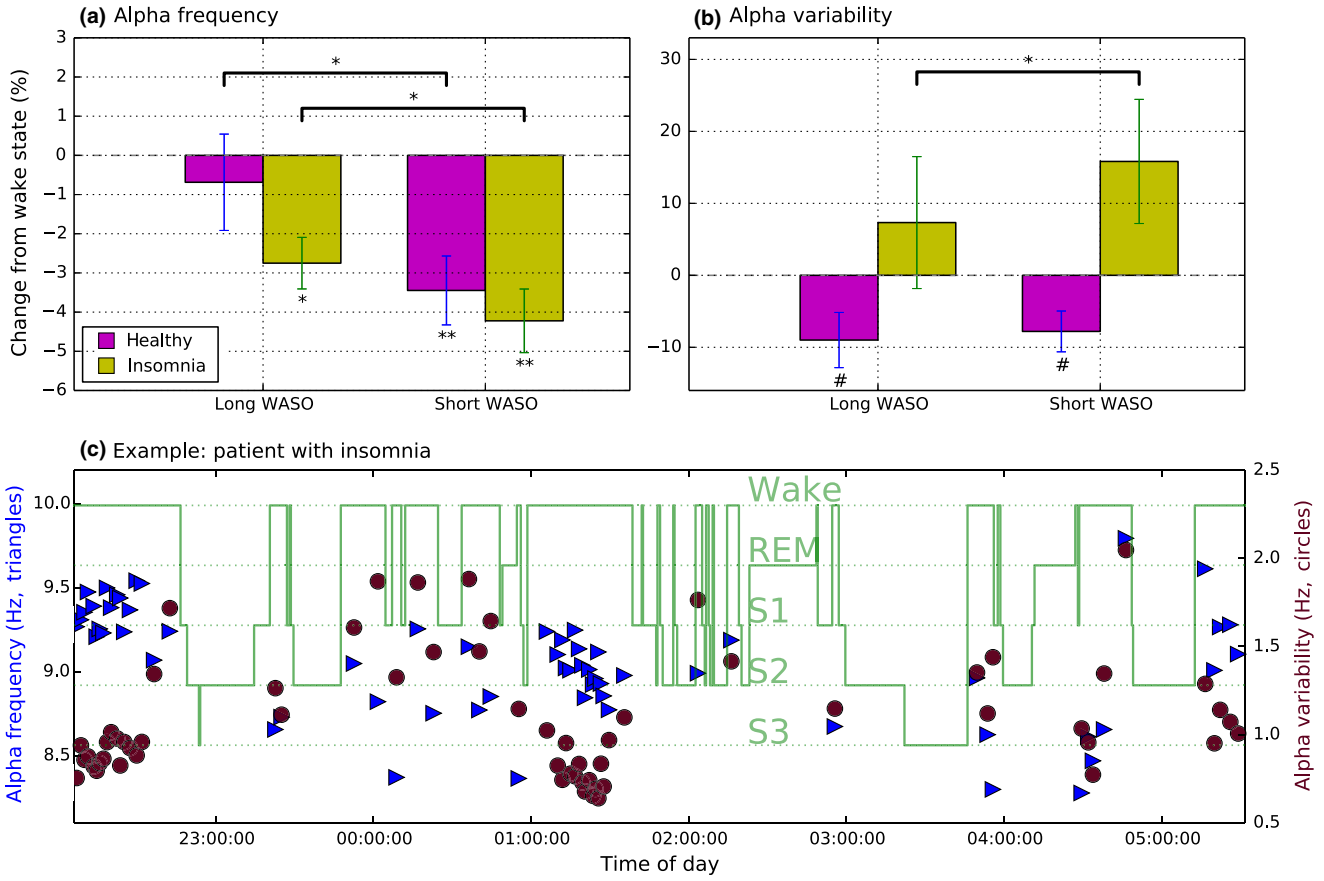

Figure 3. Change of alpha frequency and variability in wake after sleep-onset epochs (WASOs). The relative change (wrt. wake state) of alpha frequency and variability was averaged for long (>5 min) and short WASOs within the healthy and insomnia groups. (a) The frequency showed a gradual reduction from long to short WASOs for both groups alike (significance indicated). (b) The variability showed a tendency to decrease for healthy participants, but a tendency to increase for patients with insomnia that was significant for short WASOs. (c) The dynamics of alpha frequency (triangles) and variability (circles) are highlighted for a patient with insomnia together with his hypnogram (light green). Significance code: $^{**}P < 0.01$, $^{*}P < 0.05$, $^{\#}P < 0.1$ (paired Wilcoxon rank-sum test). Bar heights indicate group means, and error bars indicate their standard error.

Table 2 *P*-values of PSG parameters compared across groups of healthy sleepers and patients with insomnia

P-values	*W versus L*	*W versus S*	*L versus S*	*A versus W*	*A versus L*	*A versus S*
Healthy						
Frequency	0.6406	0.0034	0.0273	0.0015	0.0078	0.4238
Variability	0.0781	0.0803	0.3594	1.0000	0.1953	0.0269
Insomnia						
Frequency	0.0156	0.0078	0.0195	0.9375	0.1484	0.0156
Variability	0.8438	0.2500	0.0117	0.0156	0.0078	0.0156

showed an overall tendency to increase with decreasing duration of wake episodes. Specifically, short WASO alpha variability was larger than the variability of long ones ($P = 0.012$).

Healthy participants also showed a graded frequency reduction from long to short WASOs ($P = 0.027$), which was quantitatively comparable to the signature observed for patients with insomnia. However, alpha variability did not show the increase observed for the insomnia group. In fact, the healthy alpha variability showed a tendency ($P < 0.1$) to decrease for WASOs.

Alpha metrics obtained from arousals relied on only few AAIs per arousal, and therefore estimation was less reliable compared with the other modalities. After averaging, a consistent increase in alpha variability for patients with insomnia was still observed, exceeding the wake level by a median of 46 ± 17% ($P = 0.016$). The respective increase averaged across healthy participants (7 ± 7%)

did, however, not differ from their level of relaxed wakefulness.

The alpha frequency of healthy participants during these arousals was not different from the frequency observed during short WASOs ($P = 0.42$). It was reduced by $3.5 \pm 0.7\%$ from wake levels ($P = 0.0015$), and lower compared with the alpha frequency observed during long WASOs ($P = 0.008$). For patients with insomnia, the arousal alpha frequency rebounded to wake levels, and was significantly larger than in short WASOs ($P = 0.016$). All results are summarized in Table 2 and Fig. 4.

Threshold dependence of alpha metrics

The method of AAI extraction used in this work heavily relies on the amplitude threshold to determine alpha-positive segments. To illustrate the effect of the threshold, the group comparison of alpha frequency and variability in the period before sleep onset (shown in Fig. 2) was performed for a series of threshold values. Optimal threshold values were observed for each metric, at which respective *P*-values assumed a minimum. The effect was most pronounced for the alpha variability. Changing the threshold value also influenced the number of AAIs extractable from the data, as illustrated in Fig. 5b and c. At threshold 0 μV, a median of almost 10 000 AAIs were extracted; whereas at threshold 12 μV only half of the participant pool revealed the required 300 AAIs. The threshold of 8 μV was chosen as a trade-off between noise contamination and number of participants.

DISCUSSION

An analysis of alpha waves in two groups of individuals that underwent standard clinical PSG was presented: one group suffering from insomnia and one sleep-healthy group. Using a simple procedure, the alpha frequency and its variability from the PSG EEG signals were measured. These alpha metrics were compared across the PSG modalities wakefulness before sleep onset, epochs of WASOs, and short-lasting arousals of sleep.

In all participants, it was found that the instantaneous alpha frequency forms a wildly fluctuating random process with a standard deviation of about 1 Hz. These fluctuations shed a new light on earlier observations of alpha activity, wherein different alpha sub-bands were analysed (Klimesch, 1999; Scheuler *et al.*, 1990). Activity alternated between different sub-bands, which was interpreted as involvement of several processes in the alpha rhythmogenesis (Scheuler *et al.*, 1990). In the authors' view, the same rhythm-generating mechanism shows a fluctuating dynamic output (H1), which may also explain alpha activation in different frequency bands.

Furthermore, healthy and insomnia groups differed in their alpha variability before sleep onset, and showed signatures of alpha metrics across the PSG modalities that were characteristic for each group, as discussed in the following sections.

While the current results indicate a possible relevance of alpha frequency and variability in clinical sleep research, this study suffers from limitations regarding the small group of participants, of which only 1 night was analysed, as well as the novelty of the technique of alpha-wave analysis. Studies of larger cohorts are necessary to test the present results and to further investigate optimal criteria for setting the alpha amplitude threshold. Furthermore, the origin and physiological relevance of instantaneous fluctuations in the alpha frequency are not understood. The patient group suffered from severe insomnia showing strong alterations in PSG parameters (Table 2). Their reduced TST of 100 min compared with healthy participants exceeds typical values of 25 min reported by Baglioni *et al.* (2014).

Somnographic alpha characteristics

Amplitude fluctuations of alpha oscillation during resting wakefulness mark the transition to sleep. This alpha decay may be related to alpha desynchronization or a destabilization of the alpha-rhythm-generating process. Insomnia is believed to interfere with this process according to the hyperarousal model describing a tonic psychophysiological

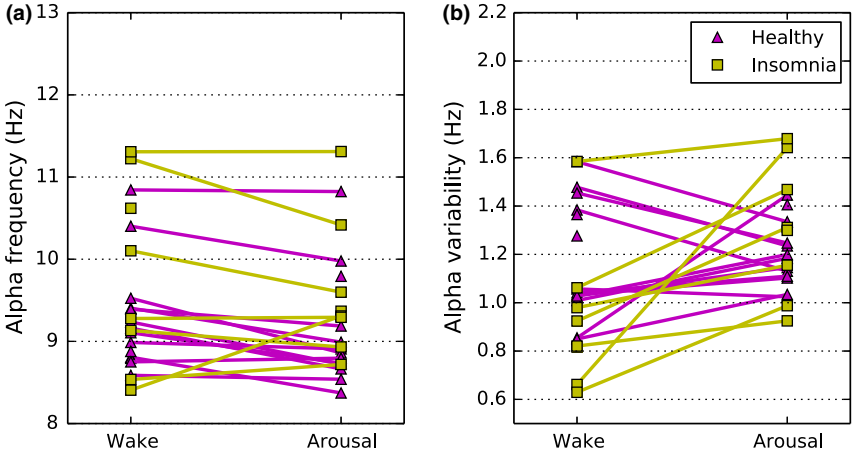

Figure 4. Comparison of alpha frequency and variability in the wake state and arousals of sleep. Alpha metrics during wake and arousals are shown for each participant (connected markers). (a) In arousals, healthy participants typically showed a decrease in alpha frequency with respect to wake ($P = 0.0015$). (b) Patients with insomnia showed an average increase of 40% in alpha variability ($P = 0.012$). For some participants, only wake alpha metrics could be obtained.

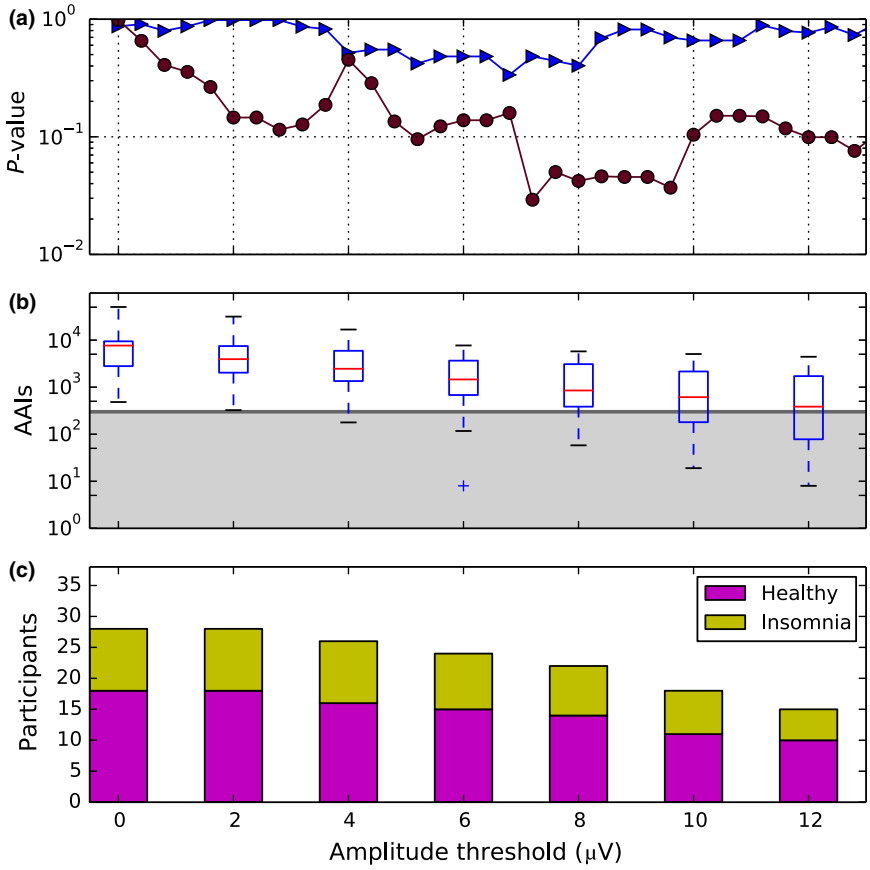

Figure 5. Threshold dependence of alpha-wave comparison across groups. (a) *P*-values of each alpha-wave metric (triangles: frequency; circles: variability) showed a minimum with respect to the amplitude threshold. (b) The number of alpha-to-alpha intervals (AAIs) found in each dataset decreased with increasing threshold. At a threshold value of 12 μV, only 300 AAIs were found in only half of the participants. (c) Consequently, the number of participants included in the statistical analysis shrunk with increasing threshold. The found minimum *P*-value at intermediate thresholds likely arose due to an interplay between noise contamination of AAIs and the number of participants included in the analysis.

activation (Riemann *et al.*, 2015), and as evidenced by EEG power spectral analysis (Freedman, 1986; Merica *et al.*, 1998; Spiegelhalder *et al.*, 2012). Conversely, patients with insomnia may demonstrate an alpha rhythm of enhanced precision (H3). In support of this hypothesis, the current data showed that alpha variability in patients with insomnia is reduced compared with that in sleep-healthy participants (cf. Fig. 2b). One may argue that hyperarousal enhances the wakefulness-promoting mechanism that in turn stabilizes the alpha frequency. This description is in line with the findings of Jin *et al.* (2006) relating vigilance assessed through visual reaction times to the width of the spectral alpha peak: the authors found narrower peaks for subjects with faster reactions. Note that it was ensured that participants in both groups were in a comparable state of relaxation by selecting those alpha waves directly before alpha decay demarcated the onset of sleep. The alpha frequency was found not to be significantly different, which was seen as evidence that indeed healthy participants and patients with insomnia were in a comparable mental state (cf. Fig. 2a). The observed difference of alpha variability may also be ascribed to the slight, but significant, group differences in age. It was noted, however, that the alpha frequency, which is known to depend on age, did not differ between the groups. Therefore, age is also believed to be a negligible factor for the alpha variability, but this remains to be tested in further investigations.

Alpha waves reappeared in the data during brief awakenings of various durations. The character of alpha waves appearing during these arousals was investigated, and in both groups a pattern of alpha frequency reduction was found, wherein short WASOs showed a lower frequency than long WASOs. This length dependence possibly indicates a correlation of alpha frequency and the microstate of mental functioning, as it transitions between sleep and relaxed wakefulness. The existence of such a spectrum of microstates was predicted by Lo *et al.* (2002), who were able to correctly model the length distribution of such brief awakenings. The current results indicate that alpha frequency may be able to track the microstate during awakenings to a certain extent. The frequency dependence further indicates an intimate connection of alpha frequency on the psychophysiological state (H2), as it has long been seen in pathological states of reduced brain functioning.

While short and long WASOs showed a signature of alpha metrics comparable across the insomnia and the healthy group, short arousals of sleep followed a different pattern. For healthy individuals, alpha frequency and variability formed essentially a continuation of long and short WASOs. The variability was increased compared with short WASOs, but it is believed that this is due to the non-stationary nature of such short arousals. In contrast, arousal alpha frequency in patients with insomnia was not different from that during relaxed wakefulness. This rebound to wake levels of alpha

frequency in insomnia may indicate a microstate of increased consciousness, which was not observed in arousals of healthy participants. It has already been argued by Feige *et al.* (2013) that short arousals from REM sleep may evoke a subjective reduction in sleep quality commonly experienced by patients with insomnia.

Advantages of frequency-based characterization of alpha waves

Power spectral analyses are able to detect insomnia-related changes in the EEG (Freedman, 1986; Merica *et al.*, 1998; Spiegelhalder *et al.*, 2012). The increased alpha, beta, sigma and gamma power found during sleep in such patients is consistent with the theory of chronic, pathological hyperarousal (Feige *et al.*, 2013; Riemann *et al.*, 2010, 2015). It was aimed to further characterize the signature of these changes, wherein the analysis was focused on EEG alpha waves.

Typically, alpha waves are characterized by a spectral peak at about 10 Hz observable at occipital and post-central EEG derivations. The peak frequency, also called individual alpha frequency, is computed as an average across spectral frequencies that are weighted by the corresponding spectral amplitudes. Changes in peak frequency are ambiguously affected by both true frequency shifts and changes in amplitude. On the contrary, the current method was designed to disentangle amplitude and frequency information to a certain degree. As demonstrated in Fig. 1, the current method was able to determine an instantaneous alpha frequency wherein amplitude information was only used to discern the presence of oscillatory activity. The metrics of frequency and variability, which were derived from this instantaneous variable, may be extended to more sophisticated tools of time series analysis (Kantz and Schreiber, 2004). Moreover, the current method allowed to compute metrics of alpha frequency and variability even from signals wherein no spectral alpha peak was identifiable.

The thresholding procedure devised in this study avoids the contamination of alpha metrics by noise, which has a disproportionate effect on AAIs. This detrimental effect was minimized by ensuring that: (i) the high- and low-frequency power of EEG artefacts were below typical alpha levels; (ii) the alpha amplitude was above a threshold; and (iii) that super-threshold alpha persisted in intervals of at least 0.3 s (~duration of three oscillations). These conditions protect the AAI estimation of low-frequency artefacts [especially condition (i)] and high-frequency noise, which would possibly alter the peak-alpha timing or produce spurious alpha-wave-like events. Accordingly, it was found that the amplitude threshold revealed an optimal value in the discriminative analysis of group differences (cf. 'Threshold dependence of alpha metrics'). The optimum likely arose because of two overlaying effects. Few AAIs are found for threshold values that approach the characteristic amplitude of alpha waves; consecutively, many of the participants were excluded. However, at low threshold values, at which all participants were included, many of the detected AAIs were heavily contaminated by noise. A distinction between the two groups was not possible based on such noisy AAIs, either. Note that the 8 μV threshold used here is in the vicinity of the 10 μV recommendation by the AASM scoring rules for EEG alpha waves. It was thus found that the right choice of threshold is essential for alpha metrics to distinguish between experimental groups and modalities. It is believed that the current finding is applicable to, and may improve, other phase-derived methods of EEG data analysis (Bashan *et al.*, 2012; Fell *et al.*, 2006; Osterhage *et al.*, 2007).

In the context of drowsiness and sleep, the EEG theta-band activity has been implied as a reliable indicator of sleepiness (Landolt *et al.*, 2004). While generally applicable, the current method of instantaneous frequency analysis will be unreliable if such oscillations are not visible as stereotypical waves in the EEG. Such theta bursts occur only episodic, and a further refinement of the method will be necessary for an application to theta-band oscillations.

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AUTHOR CONTRIBUTIONS

Concept of data analysis: JS, MR, NW, TP; selection of participants: MR, NW; execution of data analysis: JS, MR; interpretation of results: JS, TP, MR, NW; writing of manuscript: JS, TP, MR, NW.

CONFLICT OF INTEREST

None of the authors has any potential financial conflict of interest related to this manuscript.

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