REM Sleep EEG Instability in REM Sleep Behavior Disorder and Clonazepam Effects

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Abstract

Study Objectives: We aimed to analyze quantitatively REM sleep EEG in controls, drug-naïve idiopathic REM sleep behavior disorder patients (iRBD), and iRBD patients treated with clonazepam.

Methods: Twenty-nine drug-naïve iRBD patients (mean age 68.2 years), 14 iRBD patients under chronic clonazepam therapy (mean age 66.3 years) and 21 controls (mean age 66.8 years) were recruited. Power spectra were obtained from sleep EEG (central derivation), using a 2-second sliding window, with 1-second steps. The power values of each REM sleep EEG spectral band (one every second) were normalized with respect to the average power value obtained during sleep stage 2 in the same individual.

Results: In drug-naïve patients, the normalized power values showed a less pronounced REM-related decrease of power in all bands with frequency <15 Hz than controls and an increase in the beta band, negatively correlated with muscle atonia; in patients treated with clonazepam there was a partial return of all bands <15 Hz towards the control values. The standard deviation values of the normalized power were higher for untreated patients in all EEG bands and were almost completely normalized in patients treated with clonazepam.

Conclusions: The REM sleep EEG structure changes found in this study disclose subtle but significant alterations in the cortical electrophysiology of RBD that might represent the early expression of the supposed neurodegenerative processes already taking place at this stage of the disease and might be the target of better and effective future therapeutic strategies for this condition.

Keywords: REM Sleep, REM Sleep Behavior Disorder, REM sleep without atonia, Electroencephalography, Neurodegeneration, Synucleinopathy

Statement of Significance

Previous studies on the sleep EEG in patients with REM sleep behavior disorder have been able to detect changes only during NREM sleep and also the effects of clonazepam in these patients have been detected only during NREM sleep. This study shows EEG changes during REM sleep in patients with REM sleep behavior disorder, characterized by less pronounced difference from NREM sleep and increased instability, that are partially recovered after chronic therapy with clonazepam. The knowledge of the mechanisms underlying the effects of clonazepam in this disorder might prove to be essential to better understanding its physiopathology and to arrange new and more effective (neuroprotective) drug approaches.

Introduction

REM sleep behavior disorder (RBD) is a parasomnia[1] characterized by abnormal behaviors emerging during REM sleep, often causing injury.[2,3] RBD is frequently associated or precedes neurodegenerative diseases such as synucleinopathies and is regarded as an early marker and an heraldic symptom of neurodegeneration.[4-6] The term idiopathic RBD (iRBD) is used for patients without other clinical conditions.[7] The presence of REM sleep without atonia (RSWA) is the main polygraphic feature of RBD.[1]

Bedtime clonazepam, together with melatonin, is a first-line treatment for iRBD because of the response rate of close to 90%[3] and its relative safety (it is not indicated in patients with dementia, gait disturbance or obstructive sleep apnea),[8] even after years of nightly therapy;[9] however, there are no double-blind, placebo-controlled, randomized trials with clonazepam in iRBD.[10,11] Moreover, it is difficult to devise such a study in an ethically feasible manner, given the risk of recurrent injuries (and potential lethality) usually associated with iRBD.[12]

Only few studies have quantitatively analyzed the EEG changes during REM sleep in iRBD patients[13,14] and none, to our knowledge, has evaluated the eventual modifications induced by clonazepam on REM sleep EEG. The aim of this study was to analyze the differences in quantitative EEG features during REM sleep between normal controls and drug-naïve iRBD patients and another group of iRBD patients under a long-lasting regular therapy with clonazepam.

Method

Subjects and Experimental Design

For this observational study we retrospectively collected recordings that followed a standardized protocol in consecutive iRBD patients who were considered for participation in previous studies published by our groups[15-20] and whose recordings corresponded to the technical specifications reported below in the "*Nocturnal polysomnography*" section.

The diagnosis was based on the International Classification of Sleep Disorders, 3rd Edition criteria[1] for RBD. Secondary forms of RBD were excluded on the basis of historical data, neurologic examination, and encephalic MRI findings. For this study we then identified a subgroup of consecutive patients with at a video-polysomnography (vPSG) carried out when they had never been treated before with clonazepam and another subgroup with a vPSG recorded after a period of at least one year of regular and effective treatment with clonazepam (0.5-2 mg at bedtime).

None of the normal volunteers recruited had any physical, neurological or psychiatric disorder or history of sleep problems and none was taking medication at the time of recording or had ever used a neuroleptic agent or selective serotonin reuptake inhibitors, or venlafaxine.

The original studies were approved by the local ethics committees and all subjects had provided informed consent before entering the study.

Nocturnal polysomnography

Standard nocturnal vPSG was carried out which included EEG, electrooculogram, EMG of the submentalis muscle and of both tibialis anterior muscles, and ECG. Sleep signals were sampled at 128 Hz and stored on hard disk for further analysis. The sleep respiratory pattern of each patient was monitored using oral and nasal airflow thermistors and⁄or nasal pressure cannula, thoracic and abdominal respiratory effort strain gauge and by monitoring oxygen saturation. Patients with an apnea⁄hypopnea index >5 were not included. Sleep stages were scored following standard criteria[21] on 30-s epochs; since muscle atonia can be absent in RBD, REM sleep was scored without submental EMG atonia, using EEG and electrooculogram only. Onset and offset of a REM sleep period were defined according to a method specifically developed for RBD[22,23] and available only for the old Rechtschaffen and Kales sleep staging criteria.[21] Epochs containing technical artifacts or extremely elevated muscle activity causing saturation of amplifiers were carefully detected and marked for exclusion from the subsequent quantitative EEG analysis.

A quantitative analysis of the submentalis muscle EMG activity was carried out using an established automatic scoring algorithm (REM sleep Atonia Index),[15,16] which correlates significantly with the percentage of epochs of RSWA detected with the visual method by Lapierre and Montplaisir.[15,22,24] Finally, periodic leg movements were also detected and analyzed using international standard criteria.[25]

Computation and Analysis of EEG Power Spectra

For this study, the C3/A2 or C4/A1 EEG derivation was used, for each recording, sampled at 128 Hz. Sleep epochs containing artifacts were carefully excluded from the analysis, as specified above. A Fast Fourier Transform was performed with a 2-second sliding window, every second, on EEG signals from all sleep stages, after Welch windowing. Subsequently, the total absolute power (0.5-32 Hz) and that of five different EEG bands of interest was computed for each EEG epoch analyzed (delta 0.5-2.5 Hz; theta 4.5-7.5; alpha 8-11.5 Hz; sigma 12-15 Hz; beta 15.5-30 Hz). Finally, relative power values were obtained by calculating the ratio of the absolute power of each

band to the total power and then multiplying the value by 100. The relative power values obtained were then averaged for each sleep stage in all subjects.

We chose to compare the relative spectral power obtained in the different groups because of the well-known important inter-individual differences in EEG amplitude connected with several factors, such as skull thickness and sex, also previously reported in RBD patients.[13]

Analysis of REM sleep instability

Subsequently, we carried out a careful normalization of data taking the average absolute power of the different EEG bands of interest during sleep stage 2 as the reference power, in each subject. Several alternatives were evaluated but this was considered to be the best because sleep stage 2 is the sleep stage most represented in all recordings (accounting for approximately 50% of total sleep time) and it seems to be substantially unchanged in RBD.[18,26]

These average absolute power values were used as reference (V_{ref}) for the subsequent, point-by-point calculation of the normalized values (V_{norm}) of the observed absolute power values (V_{obs}) obtained in REM sleep, following the formula:

$$V_{norm} = (V_{obs} - V_{ref})/V_{ref}$$

In this way, if V_{obs} is higher than V_{ref}, a positive V_{norm} value is obtained, on the contrary, V_{norm} is negative. The mean and standard deviation of the REM sleep V_{norm} values for each EEG band were further used in this study for statistical analysis, with the first representing the normalized changes of REM sleep values vs. sleep stage 2 and the latter representing the magnitude of their variability. We will refer to these new normalized EEG values as "EEG power ratio" in the following sections of this paper.

Statistical analysis

Before running the final analyses, a power/sample size analysis was performed on the data obtained for the delta band during REM in all subjects recruited and a sample size of 11 subjects per group was found for a power 80% and alpha 0.05 or 13 subjects per group with power 80% and alpha 0.025. Between-group comparisons were performed by means of the ANOVA, followed by the post-hoc LSD test. The chi-square test was used for frequencies. Finally, the multiple regression analysis, with the calculation of the partial correlation coefficient was carried out between Atonia Index and the relative power of the different EEG bands during REM sleep. Differences were considered significant when they were below the $p<0.05$ level.

Results

Twenty-nine consecutive drug-naïve iRBD patients were retrospectively recruited (all men), as well as 14 iRBD patients under clonazepam (0.5-2 mg at bedtime) therapy (12 males and 2 females) and 21 normal controls (8 males and 13 females). The number of subjects in each group was higher than the sample size needed, according to the power analysis described above. The gender composition of the groups was evidently different (chi-square = 26.8, p <0.0001); however, this was felt to be of low impact because of the normalization of data and because no differences were found in relative power spectra between genders in normal controls.

The comparison between age and the different polygraphic sleep parameters obtained in the three groups of subjects is reported in table 1. Mean age at onset of RBD was 62.4 years (7.20 SD) in drug-naïve patients and 59.0 (4.01 SD) in treated patients (t=1.68, NS). Only clonazepam was taken by the treated group and none of the patients had comorbid conditions. Normal controls showed a higher number of awakenings, lower sleep efficiency, higher percentage of wakefulness after sleep onset, lower amount of slow-wave sleep and higher REM sleep atonia index than both untreated and treated iRBD groups, and a lower number of sleep stage shifts than iRBD patients. Drug-naïve iRBD only differed from treated iRBD because of a lower amount of sleep stage 2.

None of the comparisons made between the three groups of subjects with the absolute EEG power spectra values, during all sleep stages, was significant (supplemental table e-1). The comparison between the relative EEG power obtained for the delta, theta, alpha, sigma, and beta bands in the three groups of subjects, during each sleep stage is depicted in figure 1. Treated iRBD patients had lower delta band relative power than drug-naïve iRBD with statistical significance during REM sleep. The theta band was significantly higher in controls than in both patient groups only during slow-wave sleep. The alpha band tended to be lower in controls but the difference reached statistical significance only vs. treated iRBD in sleep stage 2 and slow-wave sleep. The sigma band was lowest in drug-naïve iRDB and statistically significant in sleep stages one, two, and slow-wave sleep. The beta band was higher in REM sleep in treated iRBD vs. drug-naïve iRBD. A positive correlation was found between the beta band and the REM sleep Atonia Index (supplemental fig. e-1).

Figure 2 shows the comparison between the EEG power ratio obtained for all EEG bands in the three groups of subjects. It is possible to note that the clear decrease in delta, alfa, and sigma bands occurring in controls during REM sleep was significantly smaller in drug-naïve iRBD. Also patients with treated iRBD showed a decrease in these bands smaller than that of controls, but not vs.

untreated iRBD and the difference was statistically significant for the sigma band. The beta band EEG power ratio was, conversely, increased in both untreated and treated iRBD vs. controls.

The average standard deviation of the EEG power ratio are shown in figure 3. Drug-naïve iRBD patients show the highest values and controls the lowest; patients with treated iRBD having intermediate average values in all cases. In particular, the average values of the EEG power ratio standard deviation obtained in patients taking clonazepam is significantly smaller than that of drug-naïve iRBD for the delta, sigma, and beta bands.

Discussion

The present study is the first clear demonstration of EEG changes during REM sleep in iRBD patients in whom EEG power spectrum seems to be characterized by a lower degree of difference from that of stage 2 (vs. normal controls) and by a higher degree of instability. Moreover, chronic treatment with clonazepam seems to be able to revert only partially these changes.

Fantini et al.[13] were the first to report that, compared with controls, untreated iRBD patients had a lower spectral EEG power in the beta band, over the occipital regions during REM sleep. However, the observed effects were of marginal statistical significance and some methodological flaws affected their results because their beta1 band (13-22 Hz) included some sigma band frequencies, with the remaining sigma band frequencies included in the alpha band (8-13 Hz). Iranzo et al.[14] tried to replicate the study by Fantini et al.[13] and extended the analysis to untreated iRBD patients with mild cognitive impairment, but used the same frequency bands. These authors only found, in REM sleep, small increases in power of the theta and beta2 bands in iRBD patients vs. controls, in the C4 EEG channel, but a decrease in the beta1 band in the O1 lead. More significant changes were found for iRBD with mild cognitive impairment. Additionally, Sasai et al.[27] found different results with iRBD patients (most of whom with mild cognitive impairment) showing decreased alpha and beta band power over the central and occipital regions in REM sleep. Massicotte-Marquez et al.[26] did not analyze REM sleep but restricted their analysis to the whole NREM sleep and found an increase in the delta band (paralleled by the increase in the percentage of slow-wave sleep) in iRBD patients. Finally, O'Reilly et al.[28] reported a lower spindle (automatically detected) density during NREM sleep in iRBD vs. controls.

Thus, the small differences reported, with marginal statistically significance, and the discrepancy between the studies indicate that a careful normalization of data is necessary when studying EEG signals as small in amplitude as those during REM sleep. Well-known sources of

inter-individual variability can disturb the analysis, such as those connected with the physical features of the volume conduction of EEG potentials (thickness of the skull and of other tissues), different between individuals and genders. A less important role can be foreseen for muscle potential contamination in normal controls, while it might assume a higher importance in iRBD patients with RSWA.

For these reasons, we preferred to use relative spectral values and found that the drug-naïve iRBD patient delta band tended to be higher than that of controls in all sleep stages; clonazepam therapy was able to reduce significantly this band only during REM sleep (fig. 1). The increase in delta band in drug-naïve iRBD vs. controls was paralleled by a decrease in the sigma band, statistically significant in all NREM sleep stages. Only during slow-wave sleep, also a significant decrease in the theta band was found in untreated iRBD patients, compared to controls. These data seem to confirm the already reported "slowing" of the EEG activity during wakefulness and sleep in RBD patients[13,14] and the relative decrease in sigma (or spindles)[28] and theta bands might be considered, at least in part, the counterpart of the relative increase in delta band in drug-naïve iRBD. The differences between untreated iRBD and controls in the beta band were not statistically significant.

Normal controls were also found to have a slightly less deep sleep architecture than drug-free iRBD patients (higher number of awakenings, lower sleep efficiency, higher percentage of wakefulness after sleep onset, lower amount of slow-wave sleep but a lower number of sleep stage shifts than iRBD patients). This is in some agreement with previous reports of a relatively preserved sleep architecture in iRBD patients who have increased slow-wave sleep[29] but also an increased sleep stage shift rate.[30]

Relative to drug-naïve iRBD subjects, patients taking clonazepam had significantly decreased delta in REM sleep, increased alpha in sleep stage 2 and slow-wave sleep, increased sigma in all NREM sleep stages, and increased beta in REM sleep only. These data seem to be in agreement with some previous findings indicating a major effect of this benzodiazepine on NREM sleep but only a minor effect on REM sleep in iRBD patients.[17,18] Although specific data on the effects of clonazepam on the spectral content of the normal sleep EEG are lacking, it is known that almost all benzodiazepines have similar acute effects involving a reduction of EEG activities <10 Hz together with an enhancement of the sigma band during NREM sleep stage 2 and slow-wave sleep and of the beta band in REM sleep and stage 1.[31,32] However, their chronic use, as in our case probably, is followed by less prominent changes that have been described to be detectable only in some part of the night (cycle).[33]

In normal controls, fast EEG activities during sleep (beta band) seem to show a behavior opposite to that of the delta band, reaching their maxima during REM sleep, when the slow-wave activity, as well as all other EEG bands with frequency below the beta range, show their minima.[34-36] This effect of REM sleep on EEG was used in our study to achieve a reliable individual normalization of the highly inter-individual variable power spectra, by calculating the degree by which REM sleep decreased or increased the power of each EEG band, taking the average value in sleep stage 2 as a reference. Sleep stage 2 has been found to be preserved in iRBD,[18,26] (also in the present study). The normalized second-by-second REM EEG power spectra values obtained in this study allowed us to go further into a detailed analysis of the REM sleep EEG of drug-naïve iRBD patients and of the effects of clonazepam therapy.

Overall, our results on the power ratio in REM sleep were well consistent with previous work on healthy volunteers.[37] With our normalized data, we observed that the evident suppression of <15 Hz EEG bands seen in normal controls was clearly less pronounced in drug-naïve iRBD patients (fig. 2). The smaller EEG power REM suppression in the delta, alpha and sigma bands in drug-free iRBD patients was partially recovered in iRBD patients taking clonazepam.

The beta band was increased by REM sleep in all groups, especially in both iRBD groups. This can be partially explained with muscle potential contamination by the RSWA in iRBD patients, as supported by the correlation between this band and the REM sleep Atonia Index (supplemental fig. e-1). Moreover, it remained unchanged also in iRBD patients taking clonazepam that has been shown to have little, if any, effect on RSWA.[17,18,22]

These data seem to indicate that a smaller difference exists in iRBD patients between NREM and REM sleep which might indicate a generally weaker REM mechanism strength. Probably, our final observation of a higher variability of the normalized EEG power values in REM sleep of drug-naïve iRBD patients, which was clearly reduced in iRBD patients taking clonazepam (fig. 3), further supports the idea that weaker neurophysiological REM mechanisms are present in these patients that undergo fluctuations higher than those observed in controls and are reduced by clonazepam. Interestingly, a significant reduction in the variability measure was observed also for the beta band in patients taking clonazepam, suggesting that at least part of the beta band increase in REM sleep in drug-naïve iRBD might not be due only to the increased muscle activity – REM sleep Atonia index has been reported to be unaffected by clonazepam,[17,18] see also table 1 – but to a true brain activity.

The REM sleep EEG instability reported here should not be confused with the decreased REM sleep stage stability in iRBD patients, indicated by an increased rate of sleep stage shifts, recently reported by Christensen et al.[30]

Descending REM-on glutamatergic neurons of the sublaterodorsal tegmental nucleus (SLD) exert a central role in generating atonia.[38] Small bilateral lesions of the SLD result in RSWA and RBD-like behaviors in animals, but do not seem to modify the amount of REM sleep; only larger lesions also affect the amount and duration of REM sleep.[39,40] Two distinct populations of SLD neurons might control REM sleep atonia and REM sleep amount; however, REM sleep has usually been reported to be unaffected in iRBD, suggesting that only the SLD cells controlling REM sleep atonia might be involved. The results of the present study indicate, on the contrary, that even if the macroscopic aspects of REM sleep are grossly preserved, REM sleep EEG structure is indeed involved in iRBD. This involvement might be connected with the suggested activation of the cortex during REM sleep (including the motor cortex) exerted by exciting intralaminar thalamocortical neurons, activated by ascending glutamatergic SLD neurons.[38]

The empirical notion that clonazepam is strongly effective in suppressing aggressive behaviors and oneiric contents, but not effective in restoring RSWA,[17,18] is in line with our findings, suggesting that the therapeutic effect of clonazepam is probably exerted by acting on supratentorial rather than subtentorial networks, reducing the negative effects of the brainstem dysfunction on the supratentorial regions, without affecting the pathogenetic core of the disease.

One limitation of this study should be mentioned because even if none of the subjects had an evident cognitive impairment, a mild cognitive impairment might have been present in some patients of both groups that might have affected, to some extent, some results.

In conclusion, the REM sleep EEG structure changes found in this study disclose subtle but significant alterations in the cortical electrophysiology of iRBD that might represent the early expression of the supposed neurodegenerative processes already taking place at this stage of the disease[6,41,42] and might be the target of better and effective future therapeutic strategies for this condition.

Disclosures

OB reports consulting for Sapio Life. GP has consulted for UCB Pharma, Jazz, and Bioproject. MM has received a grant from Vifor. The remaining authors report no conflict of interest.

Funding

This work (RF) was partially supported by a grant of the Italian Ministry of Health ("Ricerca Corrente"). MM is supported by the Swiss National Science Foundation (Grant No. 320030_144007).

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Figure Legends

Fig. 1. Comparison between the relative EEG power obtained for the delta, theta, alpha, sigma, and beta bands in the three groups of subjects, during each sleep stage. The numbers in the graph indicate the post-hoc LSD test p values obtained when the relative EEG band ANOVA was statistically significant; Data are shown as mean (bars) and S.D. (whiskers).

Fig. 2. Comparison between the EEG power ratio obtained for the delta, alpha, sigma, and beta bands in the three groups of subjects. All band ANOVAs were statistically significant; The numbers in the graph indicate the post-hoc LSD test p values. Data are shown as mean (bars) and S.E. (whiskers).

Fig. 3. Comparison between the standard deviation of the EEG power ratio (see fig. 1) obtained for the delta, alpha, sigma, and beta bands in the three groups of subjects. The numbers in the graph indicate the post-hoc LSD test p values obtained when the relative EEG band ANOVA was statistically significant; Data are shown as mean (bars) and S.E. (whiskers).

Table 1. Comparison between age and different polygraphic sleep parameters obtained in the three groups of subjects.

	1. Controls (n=21)		2. Drug-naïve iRBD (n=29)		3. Treated iRBD (n=14)		ANOVA		post-hoc LSD tests		
	mean	SD	mean	SD	mean	SD	$F_{(2,61)}$	p<	1 vs. 2	2 vs. 3	1 vs. 3
Age, years	66.8	7.24	68.2	6.46	66.3	4.88	0.520	NS			
Total sleep time, min	356.7	90.62	342.3	68.04	368.5	38.06	0.676	NS			
Sleep latency, min	23.6	25.90	28.5	48.65	16.0	8.06	0.564	NS			
REM sleep latency, min	94.0	84.75	88.7	46.81	106.0	60.02	0.341	NS			
Number of stage shifts/hour	12.2	3.93	16.6	6.52	14.3	5.07	3.923	0.025	0.007	NS	NS
Number of awakenings/hour	7.2	2.90	5.1	2.96	4.0	2.32	5.946	0.0044	0.012	NS	0.002
Sleep efficiency, %	68.3	12.55	76.5	13.62	83.5	7.85	6.744	0.0023	0.022	NS	0.0006
Wakefulness after sleep onset, %	27.3	12.20	17.1	9.89	11.5	8.75	10.623	0.0001	0.0012	NS	0.00005
Sleep stage 1, %	7.1	4.05	9.2	3.90	7.3	3.47	2.161	NS			
Sleep stage 2, %	41.3	10.89	38.5	8.53	46.6	9.14	3.393	0.04	NS	0.012	NS
Slow-wave sleep, %	10.4	7.82	17.5	7.45	17.5	6.75	6.434	0.003	0.0014		0.0076
REM sleep, %	13.9	3.98	17.7	6.99	17.2	6.59	2.573	NS			
REM sleep Atonia Index	0.94	0.039	0.73	0.203	0.78	0.154	11.136	0.000075	0.00002	NS	0.0042
PLMS index	11.0	14.33	27.2	31.60	14.9	19.66	2.895	NS			

Figure 1.

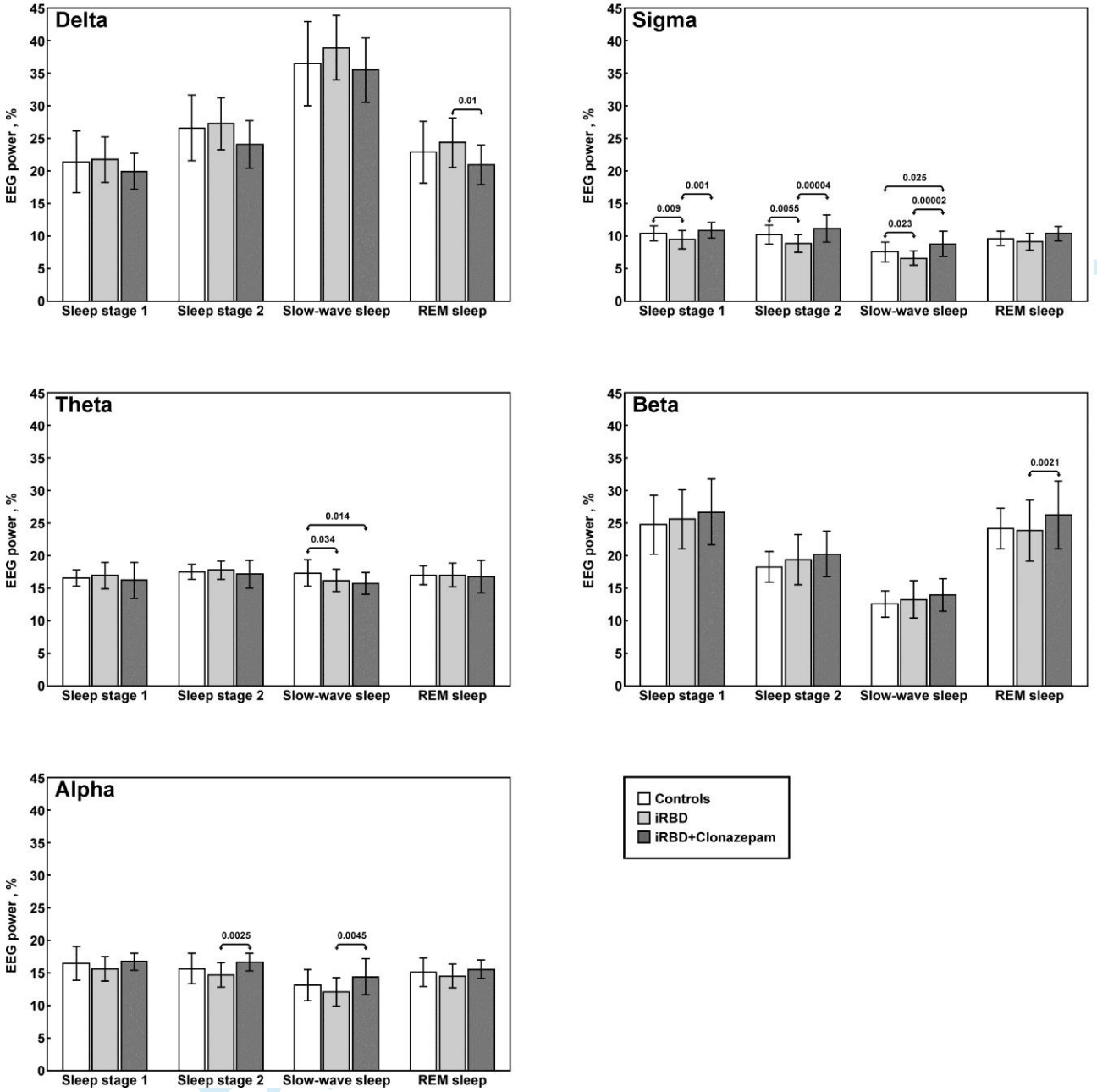

Figure 2.

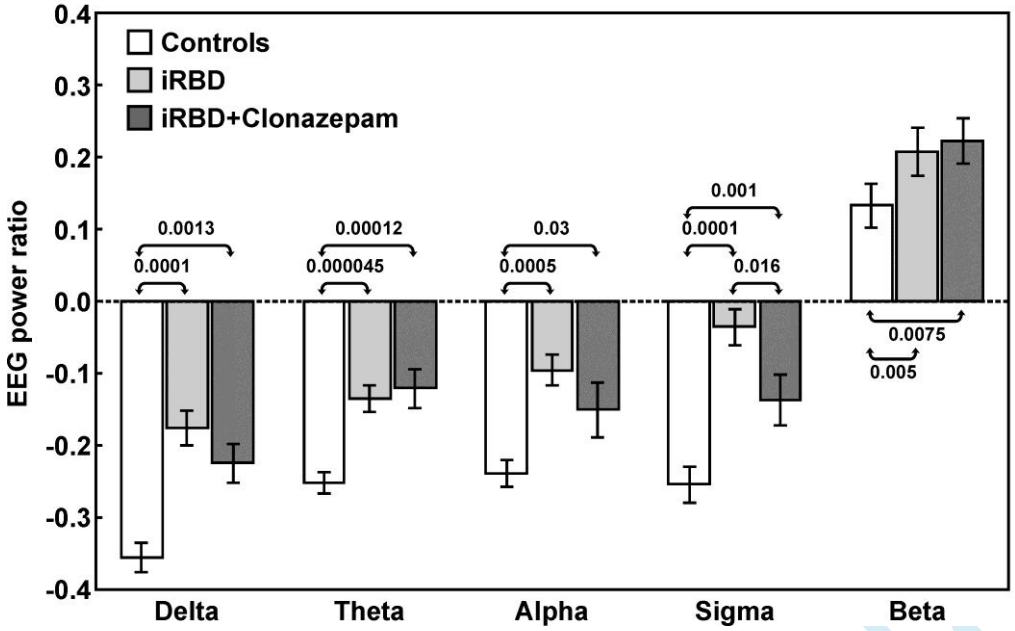

Figure 3.