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Title: Reduced delta-band modulation underlies the loss of P300 responses in disorders of consciousness

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Highlights:

- The delta and theta band modulation in response to oddball stimuli were measured in disorders of consciousness (DoC) subjects.
- DoC subjects present a reduced modulation of spectral activity in the delta band.
- There is a lack of coordinated modulation between delta and theta in response to oddball-task stimuli in DoC subjects.
Abstract

Objective: The P300 component of a sensory event-related potential is one of the major electrophysiological markers used to explore remnants of cognitive function in patients with disorders of consciousness (DoC). However, measuring the P300 in patients is complicated by significant inter-trial variability commonly observed in levels of arousal and awareness. To overcome this limitation, we analyzed single-trial modulation of power in the delta and theta frequency bands, which underlie the P300.

Methods: In a preliminary cross-sectional study using a 24-channel EEG and a passive own-name oddball paradigm, we analyzed event-related synchronization (ERS) across trials in the delta and theta bands in a sample of 10 control and 12 DoC subjects.

Results: In comparison to controls, DoC subjects presented a low percentage of trials where delta ERS was observed. In particular, coordinated modulation between delta and theta in response to the stimulus was absent, with a high percentage of trials where only theta ERS was observed. Further, we found a positive correlation between the percentage of epochs with delta ERS and the strength of the P300.

Conclusions: Reduced modulation of spectral activity in the delta band in response to stimuli indicates a dissociation in the activity of the neural networks that oscillate in delta and theta ranges and contribute to the generation of the P300.

Significance: The reduction in spectral modulation observed in DoC provides a deeper understanding of neurophysiological dysfunction and the means to develop a more fine-grained marker of residual cognitive function in individual patients.

Keywords: Vegetative state, Minimally conscious state, Electroencephalography, Delta waves, Theta waves, P300.
1. Introduction

A significant challenge in the scientific study of disorders of consciousness (DoC) is the identification of brain activity that characterizes a patient’s remnant cognitive or perceptual functions. In the vegetative state (VS), also defined as unresponsive wakefulness syndrome (Laureys et al., 2010), this identification is especially critical. VS patients are characterized by a persistent inability to express behaviors that reflect will or purpose, either spontaneously or in response to sensory stimuli. Therefore, they fail to show behavioral evidence of contact with the environment or themselves (Fernandez-Espejo and Owen, 2013, Laureys 2005). In minimally conscious state (MCS) patients, the situation is slightly different. Here, an unequivocal, albeit minimal and fluctuating, degree of connection with the environment can be verified; however, the difficulty lies mainly in the identification of the magnitude of the preserved remnant cognitive functions (Giacino et al., 2014) and the consistency with which this preservation can be measured.

Neural variables acquired through electrophysiological and neuroimaging techniques have proven useful to explore these remnant functions in DoC patients, even if the functions cannot be detected behaviorally (Coleman et al., 2009, Cruse et al., 2011, Monti et al., 2010; Owen et al., 2006). Among the neural variables extracted from EEG signals, the P300 event-related potential (ERP) has been widely used as a predictor of conscious recovery and has been associated with the preservation of remnant cognitive and perceptual functions (Cavinato et al., 2009; Kirschner et al., 2015; Schnakers et al., 2015). However, while the P300 could be useful to detect some covert abilities in a subgroup of patients under specific experimental paradigms (Bekinschtein et al., 2009; Faugereas et al., 2012), different systematic reviews have reported poor prognostic values based on this variable (Kotchoubey 2017, Kotchoubey and Pavlov 2018).

Features of the P300 reflect the continuous interplay between ongoing internal brain activity and stimulus-triggered events and the ability to generate anticipatory neural activity at successive stages of processing of the incoming sensory events (Bekinschtiein et al., 2009, Chennu et al., 2013, Polich
Given that the identification of the P300 is not always possible in DoC patients, its use as a marker of remnant functions has been difficult (Hauger et al., 2015, Real et al., 2016, Sitt et al., 2014).

Independently, the stability of neural activity in the delta and theta band is known to be valuable for identifying the P300 component (Basar-Eroglu 1992; Kolev et al., 1997; Polich 2007, Unsal and Segalowitz 1995). Though DoC patients present increased spectral power in low frequencies (Chennu 2014, Kotchoubey et al., 2005, Lechinger et al., 2013), the activity in these bands can be modulated in response to sensory stimuli (Fellinger et al., 2011; Holler et al., 2011). Spectral changes in the delta band have been related to different stages of sensory information processing and have been proposed to reflect the modulation of neural network activity subserving the transfer of information from a pre-conscious to a conscious level (Başar and Düzgün 2016). Along with their role in different cognitive functions such as working memory, changes in oscillatory activity in the theta band are related to large-scale integration through its interaction with other frequency bands (Colgin 2013, Klimesch 1999, Lisman and Jensen 2013). Thus, these coordinated spectral changes in low frequency bands (< 8 Hz) reflect the integrated activity of different neural networks relating to different perceptual and cognitive functions (Arnal and Giraud 2012, Klimesch et al., 2007).

This study aimed to apply this knowledge from cognitive neuroscience to characterize the stability of delta and theta responses generated in DoC subjects and characterize their relation to the P300 elicited alongside. Specifically, we tested the novel hypothesis that, in DoC subjects, there is a relative lack of oscillatory activity modulation at the delta and theta bands in response to stimuli, implying a loss of coordinated responses to sensory stimuli across trials, which is correlated with the lack of a P300 response.

2. Methods

2.1. Subjects
In this preliminary cross-sectional study, we recruited thirteen subjects with a diagnosis of vegetative state and minimally conscious state and ten age-matched healthy (control) subjects. The DoC subjects were recruited from hospitalized patients in the neurorehabilitation center of Los Coihues, Santiago, Chile. We evaluated brainstem auditory evoked potentials to confirm intactness of the auditory pathway. At the moment of the recording, twelve DoC subjects were receiving low doses of anti-spastic and anti-epileptic drugs, as part of their regular medications. We excluded one of the severely brain-injured subjects from the study due to excessive motion during the EEG recording, which left us with a total of twelve (4 female) subjects whose data sets were retained for further analysis. Behavioral diagnosis of patients was assigned based on the Coma Recovery Scale-Revised (CRS-R) (Giacino et al., 2004, Noé et al. 2012), which was assessed for at least six weeks before experiments started. Diagnosis, time since injury, etiology, gender, and CRS-R score are shown in Table 1.

The ethics committee of the Faculty of Medicine at the University de Chile approved all experiments and the informed consent, which was obtained from the healthy volunteers and the legally authorized representatives of the VS and MCS subjects.

2.2. EEG recording and experimental conditions

Data were acquired through a 24-channel bedside acquisition system, sampled at 1000 Hz. Two channels were allocated to register eye movements. Electrode impedances were maintained under 10 KΩ. Resting state EEG was acquired from all subjects for five minutes before the experimental task was begun. The audio signal was recorded by a Knowles microphone (K-23132) with a sampling rate of 1000 Hz, synchronized with the acquisition of the EEG signal. Onset stimuli time was defined by a detection algorithm. First, the raw audio signal was rectified and low pass filtered. The filter consisted in a convolution between a 10 ms Gaussian window and the raw audio signal, i.e., implemented a moving-mean filter. Second, we calculated a z-score of the resultant convolution vector. Third, differences between contiguous points of z-score vector was calculated. Finally, two standard deviations in the differences vector were set as a threshold to identify the stimulus onset.
After the detection of the stimulus onset, the algorithm searched for the next threshold crossing after a 1.3 sec time window. We employed an oddball task to extract ERPs and oscillatory activity (Perrin et al., 2006). The paradigm consisted of six sequences of eighty-three stimuli containing eight first names, one of which corresponded to the subject's own name (OWN) pronounced by a relative (wife/husband, mother/father or sons/daughters of each patient). The other seven first names (OFNs) corresponded to common names and were recorded by a masculine or feminine neutral voice, which matched the gender of the relative who pronounced the OWN stimulus. We programmed the task to present stimuli in a semi-random fashion, with OWN occurring at least once after five OFN stimuli (ratio OFN/OWN between 10:1 and 5:1). We delivered each OWN stimulus ten times per sequence. The inter-stimulus interval varied uniformly (i.e., same probability) between 1400 and 1700 msec. We used headphones to deliver auditory stimuli at an intensity of 80 dB. Control and DoC subjects were instructed to keep their eyes open and to be attentive to the presented stimuli. If necessary, we encouraged and stimulated DoC patients to keep their eyes open during the experiment. All subjects were laid on a comfortable chair with a 30° recline in a quiet environment.

2.3. Preprocessing and epoch selection

We implemented data preprocessing and analysis in MATLAB with EEGLAB (Delorme and Makeig, 2004) and Fieldtrip (Oostenveld et al., 2011), in conjunction with custom code. We filtered the data between 0.1 and 20 Hz and segmented it into 1500-ms epochs (first 500 ms before the stimuli). We segmented the resting state data from each participant into 200 epochs of 1500 ms. For the oddball task data, we obtained 60 epochs each in the OWN and OFN conditions. OFN epochs immediately before the OWN epochs were selected for further analysis. Each epoch was baseline-corrected relative to the mean voltage between -500 ms and stimulus onset. Epochs containing excessive noise or muscular artifact were rejected in a semi-automatic process, where outliers were defined as values larger than two standard deviations of the mean values for each channel and then rejected or retained.
by visual inspection. Independent component analysis (ICA) was used to remove ocular artifacts and muscle noise sources.

Time-frequency representations were obtained from Fourier analysis using Hanning taper with a sliding window of 500 ms in 10-ms steps. The relative change of power spectrum post onset stimuli was calculated by means of event-related synchronization (ERS) using the formula $ERS\% = \frac{(data - reference\ value)}{reference\ value} \times 100$ (Pfurtscheller and Lopes da Silva, 1999), where data corresponded to each time/frequency bin, and the reference value is the average of time/frequency bins at 500 ms before the stimuli.

2.4. Statistical analysis

2.4.1. Analysis of the rest condition

We estimated the distribution of spectral fluctuations over the 5-minute rest condition as a baseline to compare with that in task-related fluctuations. Analysis of the rest condition enabled us to determine whether the magnitude of ERS in the delta and theta band during the task were indeed related to stimulus processing, above and beyond the levels of spontaneous ERS observed during rest.

First, in each subject, we computed the distribution of time-frequency representations at the frequency of interest in midline channels (Cz, Fpz, Pz) by randomly selecting 60 of the 200 epochs in the resting condition, (60 epochs x 3 channels x 20 bins of frequency x 201 bins of time). Second, we reduce the data to two dimensions, with channels and frequency averaged separately in the delta (1-4 Hz) and theta (4-8 Hz) bands. Thus, for further analysis we kept two matrices of 60 epochs x 201-time bins (Figure 2A). To estimate relative changes in spectral power at rest, each randomly selected epoch was divided into an initial baseline period of 500 ms, relative to which we calculated the ERS in the following 1000 ms immediately after this baseline.

To address potential bias in the criterion used to select epochs, we calculated the percentage of those epochs where the increment of spectral power was greater than 50% of the baseline and sustained for at least 250 ms (25 adjacent time/frequency bins) and 130 ms (13 adjacent time/frequency bins) for
the delta and theta band respectively (these time periods were at least half a cycle at 2 and 5 Hz). We chose this threshold of 50% based on the ERS values reported at the group level in previous studies (Klimesch et al., 2007) and analyzed the magnitude of relative ERS in the rest condition between 50 and 850% in steps of 100%. Thus, we obtained the percentage of epochs showing ERS > 50% in the delta and theta band separately. Third, this procedure (first and second step) was repeated 1000 times to generate a distribution of the percentage of epochs showing relative changes in oscillatory activity at resting state for each frequency/channel/time between 1 and 8 Hz (Figure 2A). The two-sample Kolmogorov-Smirnov test was used to evaluate the hypothesis that the distributions thus generated for control and DoC subjects were statistically distinguishable ($\alpha = 0.05$).

2.4.2 Percentage of epochs showing ERS in response to stimuli

We tested for normality of the data using the Shapiro-Wilk test. For hypothesis testing, we calculated a mixed ANOVA to explore the interaction between group and stimuli. Within-subjects factors were stimuli with three levels (OWN, OFN and REST), and the between-subjects factor were groups (Control and DoC). Percentage of epochs with ERS>50% in the delta, theta, and delta/theta band were the dependent variable. Mauchly’s test of sphericity was used to test the null hypothesis that the variances of the differences are equal, and Greenhouse-Geisser correction was applied if necessary. We conducted post hoc analyses using t-tests and false discovery rate (FDR) to control for multiple comparisons. Based on the ERP grand average of the control group, the time window to estimate the amplitude of the P300 component went from 210 ms to 310 ms post-stimulus presentation. In subjects with time clusters of significant differences between conditions, we estimated the P300 peak amplitude by searching for the most positive value inside the significant time window. In subjects without significant differences, we estimated peak amplitude by searching for the most positive value in the same time window. The amplitude was calculated as the difference between this maximum positivity and zero. For DoC subjects, we allowed the possibility of a delayed response and the time window went from 210 ms to 600 ms post-stimulus presentation. The correlation between the P300
component and percentage of epochs showing ERS at the delta or theta band was estimated with Spearman’s rank correlation coefficient (rho).

2.4.3 Event-related potentials and event-related synchronization

To evaluate the hypothesis at the single-subject level that the experimental conditions produce different responses, we used non-parametric statistical testing to calculate the probability distribution obtained with the Monte Carlo method (permutation test) (Maris and Oostenveld 2007). For this, OWN and OFN trials were grouped in one set and divided into two subsets by random partitioning. Channel-time samples or channel-frequency-temporal samples estimates from both subsets were compared with a two-sided t-test. We grouped all the samples whose t-value was larger than the critical alpha level (0.05) by a temporal adjacency criterion. The sum of all t-values in each cluster was saved, and the clusters with the maximum and minimum value were conserved. We repeated the procedure 1000 times to generate the non-parametric null distribution and determined the p-value based on the proportion of the distribution resulting in a larger test statistic than that observed in the original OWN and OFN trials. If this non-parametric cluster-level p-value was smaller than the critical alpha-level (0.05), we rejected the null hypothesis, thus accepting the alternative hypothesis that the data came from different distributions and were significantly different. In line with previous reports describing the evoked activity in the midline channels (Fpz, Cz, Pz), we used the average activity of these channels for spectral analysis and the Cz channel to estimate the relationship between the P300 component and percentage of epochs with ERS>50% in the delta and theta band.

3. Results

3.1 Event-related and low frequency responses at the group level

For ERS in the OWN (own name) and OFN (other first name) conditions, we found that there were no differences in DoC patients (Figure 1A). In controls, there were key differences in the ERS between the OWN and OFN conditions: in the delta band between 230 ms and 540 ms and in the theta band between 400 and 520 ms (permutation test, N=10, \text{clusterstat} = 531.4, \text{p} = 0.011,
Simultaneously, we observed event-related activity in the control and DoC groups (Figure 1B). In the DoC group, there were no significant differences in responses to OWN and OFN stimuli. In the control group, we observed differences in the ERP between 111 and 185 ms (permutation test, N = 10, clusterstat = -282.03, p = 0.022, corrected; cluster-forming threshold $P < 0.05$) and between 212 and 306 ms between the OWN and OFN stimuli (permutation test, N = 10, clusterstat = 384.13, p = 0.002, corrected; cluster-forming threshold $P < 0.05$) (Figure 1B). These results confirm previous research showing the lack of a differentiated response between stimuli at the group level in DoC subjects.

Differences between controls and DoC patients in delta and theta modulation were absent at rest. At rest, the average percentage of epochs for the midline channels showing fluctuation in power spectral activity (50-850%) did not differ between the control and DoC group in the delta band ($Z_{KS} = 0.236; D = 0.111; p = 1$) (Figure 2B) or theta band ($Z_{KS} = 0.236; D = 0.111; p = 1$) (Figure 2C).

Thus, at rest, the magnitude of spectral power fluctuations for the delta and theta band was similar between control and DoC subjects.

### 3.2. Delta modulation at the single-subject level

Trial-by-trial analysis at the individual level allowed us to identify the presence of ERS in the delta and theta band for both stimuli in all DoC and control subjects. Figure 3A shows an example of a DoC subject with a low percentage of epochs with ERS > 50% (OWN/OFN: 29/36% for delta and 52/55% for theta, DoC6 at channel Cz), which was not evident in the average (3A right). In contrast, Figure 3B shows an example in which there was a high percentage of epochs with ERS > 50% in a control subject (CS2; OWN/OFN: 67/43% for delta and 73/58% for theta). In the single-subject averages, we observed ERS > 50% at delta and theta bands for all control subjects, but only six of these subjects showed differences between OWN and OFN in the range of 50-600 ms (permutation test at the single-subject level, N = 60, clusterstat between 671.05 and 1539.2, p < 0.0001 for CS2, CS5, CS7, CS8; and clusterstat between 295.1 and 404.3, p < 0.0015 for CS1 and CS3, corrected;
cluster-forming threshold $P < 0.05$). In the DoC group, we observed only one subject (DoC1) who showed ERS differences between the OWN and OFN conditions (permutation test at the single-subject level, $N = 58$, clusterstat = 310.4, $p = 0.03$, corrected: cluster-forming threshold $P < 0.05$). These results suggest that despite there being no differences in the average, the identification of delta and theta modulation in response to stimuli is possible at individual trials.

Similarly, we observed ERPs in all control subjects, seven of whom showed differences in the P300 component between OWN and OFN trials (permutation test at the single-subject level, $N = 60$, clusterstat between 290.17 and 608.35, $p < 0.005$ for CS1, CS2, CS3, CS5, CS7, CS8, CS 10, corrected; cluster-forming threshold $P < 0.05$). Two control subjects did not present differences between conditions (CS4, CS6) (permutation test at the single-subject level, $N = 60$, $p > 0.05$, corrected: cluster-forming threshold $P < 0.05$). Importantly, these were the subjects with the lowest percentage of epochs showing delta and theta ERS. In the DoC group, four subjects showed an ERP component (DoC1, DoC3, DoC4, DoC8), two of which showed differences between OWN and OFN (permutation test at the single-subject level, DoC 4: $N = 59$ clusterstat = 187, $p = 0.016$; DoC 8: $N = 56$, clusterstat = 129.3, $p = 0.031$, corrected: cluster-forming threshold $P < 0.05$).

For the control group, there was a larger percentage of epochs showing ERS > 50% in the delta band than in the DoC group for OWN and OFN stimuli at midline channels (Figure 3C), which is reflected in a main effect of stimuli ($F (2,19) = 11.785$, $p < 0.0001$). In the control group, post hoc t-tests revealed differences between OWN and OFN stimuli ($t (9) = 4.14$, $p = 0.006$), OWN and REST ($t (9) = -5$, $p = 0.006$) and OFN and REST ($t (9) = 2.89$, $p = 0.021$). In the DoC group, the post hoc tests revealed differences between OWN and REST ($t (11) = -3.53$, $p = 0.0075$) and OFN and REST ($t (11) = -3.89$, $p = 0.006$) but not between OWN and OFN ($t (11) = 0.82$, $p = 0.42$). At the same time, the percentage of epochs showing ERS > 50% was larger for the control than for the DoC group, with a highly significant main effect of group ($F (2,20) = 54.6$, $p < 0.0001$) and a significant interaction (Group x Stimulus; $F (1,20) = 54.6$, $p < 0.0001$). Post hoc t-tests revealed differences in the percentage
of epochs showing ERS > 50% between groups in the response to OWN (t (20) = 6.85 p < 0.0001) and OFN stimuli (t (20) = 5.044, p < 0.0001), but no such differences between groups were observed for the REST condition (t (20) = -0.326, p = 0.748). In other words, DoC subjects showed a decrement in the modulation of neural activity in the delta band in response to stimuli compared to control subjects.

3.3. Theta modulation at the single-subject level

In contrast to the delta band, midline channels showed no differences in the percentage of epochs presenting ERS > 50% at the theta band (Figure 3D bottom), with no main effect of stimuli (F (2,40) = 3.11, p = 0.055). At the same time, the percentage of epochs showing ERS>50% in the theta band was larger in the control group than in the DoC group, with a main effect of group (F (1,20) = 20.64, p < 0.0001) and a significant interaction (Group ´ Stimulus; F (1,20) = 16.1, p = 0.001). Post hoc t-tests revealed differences across groups in response to OWN (t (20) = 3.72, p = 0.001) and OFN stimuli (t (20) = 3.63, p = 0.002), but these differences were not observed in the REST condition (t (20) = -0.708, p = 0.48). Thus, control subjects generated a selective response to OWN stimuli mainly in the delta band. In contrast, the DoC group did not show this differentiated response in the delta or theta band. However, DoC subjects showed an increase in the percentage of epochs showing ERS in the theta band in comparison with that in the delta band for OWN (t (11) = 11.26, p = 0.0001) and OFN stimuli (t (11) = 12.01, p = 0.0001).

3.4 Delta/theta response

Having characterized the trial-by-trial fluctuations present in the DoC subjects, we explored the interaction between responses in the delta and theta frequency bands. We first identified separately for the midline channel the epochs in which delta was above 50% and epochs in which theta ERS was above 50%. We then selected only those epochs in which there was a coordinated response in both bands (i.e., delta and theta ERS both above 50% in that epoch). In the control group, the modulation of oscillatory activity in response to stimuli showed differences between OWN and OFN when
selecting epochs with delta ERS > 50%, ) (permutation test at group level, N = 10, clusterstat = 479.4
p = 0.006, corrected: cluster-forming threshold $P < 0.05$) epochs with theta ERS > 50% (permutation
test at group level, N = 10, clusterstat = 665.6 p = 0.008, corrected: cluster-forming threshold $P <$
0.05) , or epochs with delta and theta ERS > 50% (permutation test at group level, N = 10, clusterstat
= 534.81 p = 0.008, corrected: cluster-forming threshold $P < 0.05$) (Figure 4A, 4B and 4C, right). In
contrast, in the DoC group, there were no differences between stimuli in epochs with ERS > 50% in
the delta or theta bands or in the delta and theta together (Figure 4A, 4B, and 4C, left). Time-frequency
plots of DoC subjects showed that the epochs with theta-band ERS did not produce delta-band ERS
alongside. (Figure 4B, left).

In the control group, there was a larger percentage of epochs showing ERS > 50% in the delta and
theta bands simultaneously than in the DoC subjects, which was reflected in the main effect of stimuli
(F (2,20) = 4.827, p = 0.039). Further, post hoc t-tests in controls revealed differences in this
percentage between OWN and OFN stimuli (t (9) = 2.42, p = 0.039), OWN and REST (t (9) = 4.6, p
= 0.022) and OFN and REST (t (9) = 3, p = 0.003). In the DoC group, post hoc t-test showed
differences between the OWN and REST conditions (t (11) = -2.86, p = 0.045) but not between OWN
and OFN (t (11) = -0.037, p = 0.971) or between OFN and REST (t (11) = -2.218, p = 0.0735). Further,
the percentage of epochs showing ERS > 50% was larger in controls than in DoC subjects, with a
highly significant main effect of group (F (1,20) = 28.75 p < 0.0001) and interaction (Group x stimulus
(F (1,20) = 32.331, p < 0.0001). Post hoc t-test showed differences between groups in the percentage
of epochs registering ERS > 50% during the response to OWN stimuli (t (20) = 5.4, p = <0.0001) and
OFN stimuli (t (20) = 3.615, p = 0.002) but not during REST (t (20) = -0.8, p = 0.428). At rest, midline
channels (FCz, Cz, Pz) showed no differences in the percentage of epochs presenting ERS > 50% at
the delta and theta band, with no main effect of stimuli (F (2,19) = 1.052, p = 0.369).

Considering the epochs showing ERS > 50% in the delta and theta bands simultaneously, we
estimated the ratio of percentage of ERS modulation in delta/theta. Midline channels (FCz, Cz, Pz)
showed no differences in the ratio delta/theta ERS for OWN stimuli, with no main effect of stimuli (F (2,19) = 3.273, p = 0.06). At the same time, controls and DoC subjects showed no main effect of group (F(1,20) = 3,404, p = 0.08). Similar results were founded of OFN stimuli, midline channels showed no differences in the ratio delta/theta ERS for OFN stimuli, with no main effect of stimuli (F (2,19) = 8.30, p = 0.451), and controls and DoC subjects showed no main effect of group (F(1,20) = 1.058, p = 0.24).

When considering the percentage of epochs showing ERS > 50% only in the theta but not the delta band, we found differences between the control and DoC groups in the percentage of epochs with ERS > 50% in response to OWN stimuli (t (20) = -5.117, p < 0.0001). Interestingly, these differences were not observed for the OFN stimuli (t (20) = -2.279, p = 0.068) (Figure 5A). We did not observe differences between groups in the percentage of epochs with ERS > 50% only in delta (but not in theta) (Figure 5A) in the responses to either OWN (t (20) = -1.08, p = 0.845) or OFN stimuli (t (20) = -0.198, p = 0.39). Taken together, this pattern of results suggests a lack of coordinated response in low frequency bands in DoC subjects, with many epochs changing only in the theta band in response to the stimulus.

3.5. Single-subject correlations between P300 and delta modulation

In the individual subjects where it was possible to identify ERPs (all controls and DoC subjects 1, 3, 4, 8), we identified a strong correlation between the peak amplitude of the P300 component and the percentage of epochs that show ERS > 50% in the delta band (RHO = 0.82, p=0.0003) (Figure 5B) but not in the theta band (RHO = 0.23, p=0.42). It is relevant to note here that the correlation was computed considering different numbers of control and DoC subjects.

Overall our results provide clear evidence that the coordinated response across delta and theta bands is absent in subjects with DoC, who show reduced modulation in the delta band. Thus, these results are consistent with a lack of integrated activity at low frequency bands. At the same time, our findings
demonstrate that the P300 amplitude covaries with the percentage of epochs showing ERS in the delta band but not in the theta band.

4. Discussion

In this study, we explored the coordination of delta- and theta-band modulation in response to auditory stimuli and their relationship with the ERP P300 component in patients with disorders of consciousness. Due to the relevance of low-frequency oscillatory activity to the P300, we carried out analyses of the trial-by-trial response in the delta and theta bands at the single-subject level. In patients, we found a lack of coordinated response across low frequency bands, mainly determined by a decrease in the stimulus-triggered delta-band response. At the same time, we found a significant correlation between the percentage of epochs showing ERS in the delta band and the P300 amplitude. From a spectral frequency perspective, the P300 component is mainly determined by phase-locked oscillatory activity in delta and theta bands (Polich 2007, Stampfer and Başar 1985). Previous reports have shown an association between increased delta- and theta-band power and the amplitude of the P300 component (Başar -Eroglu et al., 1992). Exploring the stability of oscillatory responses at the single-trial level in individual subjects addresses a relevant and important challenge in the assessment of subjects with DoC, i.e., the significant variability in response from one trial to the next. We showed that, compared to controls, subjects with DoC showed a reduced number of epochs with stimulus-triggered delta-band ERS. It has been suggested that the increase in the spectral power of the delta oscillatory activity in response to different stimuli allows the normal transitions between different states of consciousness (Başar and Düzgün 2016). At the same time, the modulation of delta oscillations could have a key role in processes related to input selection, expectation, and integration across different frequencies (Lakatos et al., 2005, Schroeder and Lakatos 2009).

The theta-band response has also been related to different mechanisms of cognitive processes and to different encoding and retrieval processes of events in memory (Cavanagh and Frank 2014, Klimesch 2007). In DoC, theta-band ERS increases have been reported when patients perform a task where they
were asked to detect and count instances of their own name (Fellinger et al., 2015). We did not find differences in the theta band in the control group between the OWN (own name) and OFN (other first names) conditions, which was probably due to the passive nature of our task.

For DoC subjects, we found a high percentage of epochs with a response in the theta band without modulation in the delta ERS (Figure 5A). This result reinforces the idea that the changes in delta activity relate to the ability to consciously engage with external stimuli. Is necessary to consider that in this work the own name stimulus is presented in a uniquely-different voice, probably adding novelty outside the own-name characteristic. Novelty features of the stimuli are related with a P3a component elicited with a short peak latency (Polich 2007), and in the experimental design used here could be related with the early P300 peak observed in our results (Figure 1B). At the same time, previous reports have described the relevance of modulations in delta and theta activity to salient stimuli and emotional processing (Knyazev 2012).

Changes in the magnitude of oscillatory activity are inherent in different brain rhythms and could influence the ability to process different stimuli at different frequency bands (Addante et al., 2011, Hanslmayr et al., 2011, Palva and Palva 2007). Interestingly, the distribution of these fluctuations at rest was similar in the control and DoC groups (Figure 2). In contrast, the DoC group showed a significant decrease in the percentage of epochs showing ERS at delta and theta bands in response to stimuli. Spontaneous EEG activity in subjects with DoC and other pathological conditions is characterized by an increase in spectral power in low frequency bands (Chennu et al., 2014, Kotchoubey et al., 2005, Lechinger et al., 2013, Lehembre et al., 2012). In contrast to the neural networks in controls, neural networks oscillating in the delta range in DoC subjects might be unable to entrain their activity in response to stimuli (Stefanics et al., 2010). If this is the case, the loss of modulation in the delta band could be affecting the hierarchical control over the activity of networks oscillating at higher frequencies, in particular in the alpha band.
The contribution of spectral power changes to the generation of the P300 component is only one aspect by which oscillatory activity is modulated by sensory processing. **We are aware that the high correlation value between the percentage of epochs showing ERS and the amplitude of the P300 cannot be taken as evidence of a causal relationship between both variables.** In this way, other aspects as the distribution of the phase in the oscillatory activity over the selected periods have not explored in this work, which could be a topic of further research.

Using the conceptual framework from the literature describing the role of oscillatory activity in delta and theta bands, new research questions may be raised. Because delta-band responses can be divided into an early component related to bottom-up processing and a late component related to top-down processing (Başar and Düsgün, 2016), these two components may be experimentally dissected in subjects with DoC. On another note, changes observed in single trials may reflect transient changes in neural activity related to fluctuations in the ability to process sensory stimuli. Active paradigms may generate changes in the magnitude, phase, and stability of spectral changes that may facilitate detection of remnant abilities. Crucially, an advantage of the trial-by-trial analysis in the frequency domain in low bands, as we have conducted here, is that this method allows us to detect changes in the electrical activity of the brain in response to different stimuli that cannot be observed in the time domain average.

**5. Conclusions**

Our exploration of the spectral features of neural responses contributes to a more detailed characterization of the changes in sensory processing in DoC. Drawing upon current views of loss of coordinated activity in DoC (Laureys 2005, Schiff 2010), our preliminary results suggest that patients show a lack of coordinated spectral modulation in delta and theta bands in response to auditory stimuli. In addition, this decrement in delta-band modulation is related to the generation of the P300. Our findings shed light on key pathological alterations in DoC and contribute novel fine-grained markers of remnant cognitive function.


Figures Captions

**Figure 1. Time frequency and event-related response at the group level.** Spectral change and event-related activity in response to auditory stimuli. A-. Time-frequency representation, DoC group (top) and Control group (bottom); areas circled in black show significant differences. B-. Event-related responses to OWN (green) and OFN (red); gray line at bottom shows time bins in which significant differences were identified.

**Figure 2. Estimation of ERS during resting state period and delta-theta relative changes.** A-. Data analysis procedure to estimate the distribution of ERS changes at rest. B-. Distribution of the percentage of epochs changing at different ERS magnitude in the delta band, during resting state. C-. Distribution of the percentage of epochs changing at different ERS magnitude in the theta band, during resting state. In B and C, each dot corresponds to one subject. DoC, blue circle; Control, green circle.

**Figure 3. Relative spectral changes (ERS) in response to auditory stimuli.** A-. Example of trial-to-trial ERS variability of a DoC subject for delta and theta band (DoC5) and his average time frequency representations. B-. Example of trial-to-trial ERS variability of a control subject for the delta and theta band (CS9) and his average time frequency representations. Areas circled in black show significant differences between OWN and OFN. Time frequency representations and topoplot show change relative to -500 ms of baseline C-. Percentage of epochs with ERS > 50% in the delta band for OWN and OFN stimuli and at rest. D-. Percentage of epochs with ERS > 50% in the theta band at the single-subject level for OWN and OFN stimuli and at rest.

**Figure 4. Oscillatory activity at the delta, theta and delta/theta band.** A-. Time frequency representations of epochs showing ERS > 50% in delta in the control and DoC group. B-. Time frequency representations of epochs showing ERS > 50% in theta in the control and DoC group. C-. Time frequency representations of epochs showing ERS > 50% in delta/theta in the control and DoC group. Area indicated by the black line shows significant differences between OWN and OFN
conditions. D-. Percentage of epochs showing ERS > 50% in both delta and theta simultaneously in the DoC and control subjects.

**Figure 5. Decrement in delta responses and the P3 component.** A.-Percentage of epochs with ERS > 50% in theta but not delta in DoC subjects (blue) and control (green) (left). B.- Correlation between percentage of epochs with ERS > 50% in the delta band with pick of ERP between 200-400 ms (C: control subjects; D: DoC subjects).

**Table 1**

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Table 1. Clinical and demographics features of patients with DoC. Diagnostic, time since injury, etiology, gender, and CRS-R score for DoC subjects.