Restorative Neurology and Neuroscience 32 (2014) 767–787
DOI 10.3233/RNN-140426
IOS Press

A case of post-traumatic minimally conscious state reversed by midazolam: Clinical aspects and neurophysiological correlates

Maria Chiara Carboncini[a,*], Andrea Piarulli[b], Alessandra Virgillito[a], Pieranna Arrighi[a], Paolo Andre[d], Francesco Tomaiuolo[c], Antonio Frisoli[b], Massimo Bergamasco[b], Bruno Rossi[a] and Luca Bonfiglio[a]
[a]*Department of Neuroscience, University of Pisa, Pisa, Italy*
[b]*PERCRO (Perceptual Robotics) Laboratory, Scuola Superiore Sant'Anna, Pisa, Italy*
[c]*Auxilium Vitae, Volterra, Pisa, Italy*
[d]*Department of Medical and Surgical Sciences and Neuroscience, University of Siena, Siena, Italy*

Abstract.
Purpose: We describe the case of a subject in a post-traumatic Minimally Conscious State (MCS) who retrieved full interaction with the environment after midazolam infusion. We studied EEG correlates of the "awakening reaction" in the different domains of frequency, time and cortical topography, along with the intrinsic connectivity within both the task-positive and the linguistic network.
Methods: EEG recorded before and after midazolam administration has been submitted to spectral power analysis, sLORETA analysis and intrinsic connectivity analysis within both functional networks.
Results: A critical change in the power spectrum profile was observed after midazolam: a) the power between 1 and 12 Hz decreased, reaching its maximum difference with respect to pre-infusion at about 7 Hz and b) the power between 12 and 30 Hz increased, with a maximum difference at about 15 Hz. At the same time, midazolam induced significant connectivity changes, especially for these two frequency bands, within both functional networks.
Conclusions: We advance some hypotheses about certain aspects of the recovery from the MCS both in terms of anatomo-functional correlations and functional brain systems and we make inferences about the role that some kind of 'catatonic' symptoms might play in determining and/or maintaining this peculiar clinical state.

Keywords: Connectivity, catatonia, disorders of consciousness, EEG, GABAergic drugs, minimally conscious state, non-convulsive status epilepticus, theta rhythm

1. Introduction

To our knowledge, no reporting has been made regarding the "awakening" effect of midazolam.

Herein we describe the case of a subject in apparent minimally conscious state, who retrieves the full interaction with the environment after midazolam administration.

*Corresponding author: Dr. Maria Chiara Carboncini, Department of Neuroscience, University of Pisa, via Paradisa 2, 56126 Pisa, Italy. Tel.: +39 50995581; Fax: +39 50995723; E-mail: m.carboncini@ao-pisa.toscana.it.

As a result of a car accident SV, a 43-years-old male, had a craniofacial trauma. He was admitted to the hospital scoring 4 to the Glasgow Coma Scale (GCS); the CT scan at the admittance indicated the presence of little contusive hemorrhagic lesions into the right temporo-insular region and in the left white matter around the posterior horn of the lateral ventricles. SV had been in coma for 40 days and subsequently in vegetative state for four weeks. He further evolved after 180 days turning in Severe Disability status according to Giacino classification criteria (Giacino et al., 2002). He was dismissed after ten months. At discharge he

had psicomotor slowness, lack of verbal initiative and dependence in all life activity. Behind insistence he was able to read aloud single words and numbers.

After about 1 year from trauma, because of diurnal sleepiness onset, hydrocephalus was suspected, thus he underwent a further CT scan. Since a dilatation of ventricular volume was detected, a ventriculo-peritoneal shunt was positioned. Soon after the shunt, SV went back to the previous status (i.e. psychomotor slowness and lack of verbal initiative). A progressive cognitive ability decline occurred, as verified during the medical examination for the annual shunt control two years after trauma: SV did not produce any single word, he did not answer even to very simple requests (e.g. close your eyes) and did not communicate in any way. The general physical examination let on psychomotor bradykinesia, amimica facies, reduction of the blink rate, increased muscle tone plastic type and no limbs weakness. He had enlargement of the support base, overall body rigidity and bradykinetic gait. He produced aimless repetitive behaviours (e.g., clapping) and resistance to passive motion proportional to the strength of the stimulus.

He underwent EEG, P300, PET and CT. The EEG pattern revealed a widespread theta rhythm and an amplitude reduction of P300 response was observed when registering acoustic evoked potentials. A F18-FDG PET revealed a reduction of the metabolic activity in the overall cingulate, frontal and in the temporal cortex bilaterally, larger into the right hemisphere. During this period we attempted different drug therapies testing individually their effect. We tested amantadine, L-dopa and zolpidem. None of the tested drugs resulted in SV clinical status modification, thus we decided to suspend any kind of drug administration.

He underwent CT scan for the routinary shunt control (see Fig. 1) and, in order to avoid motion artifacts, for the first time SV was mildly sedated using

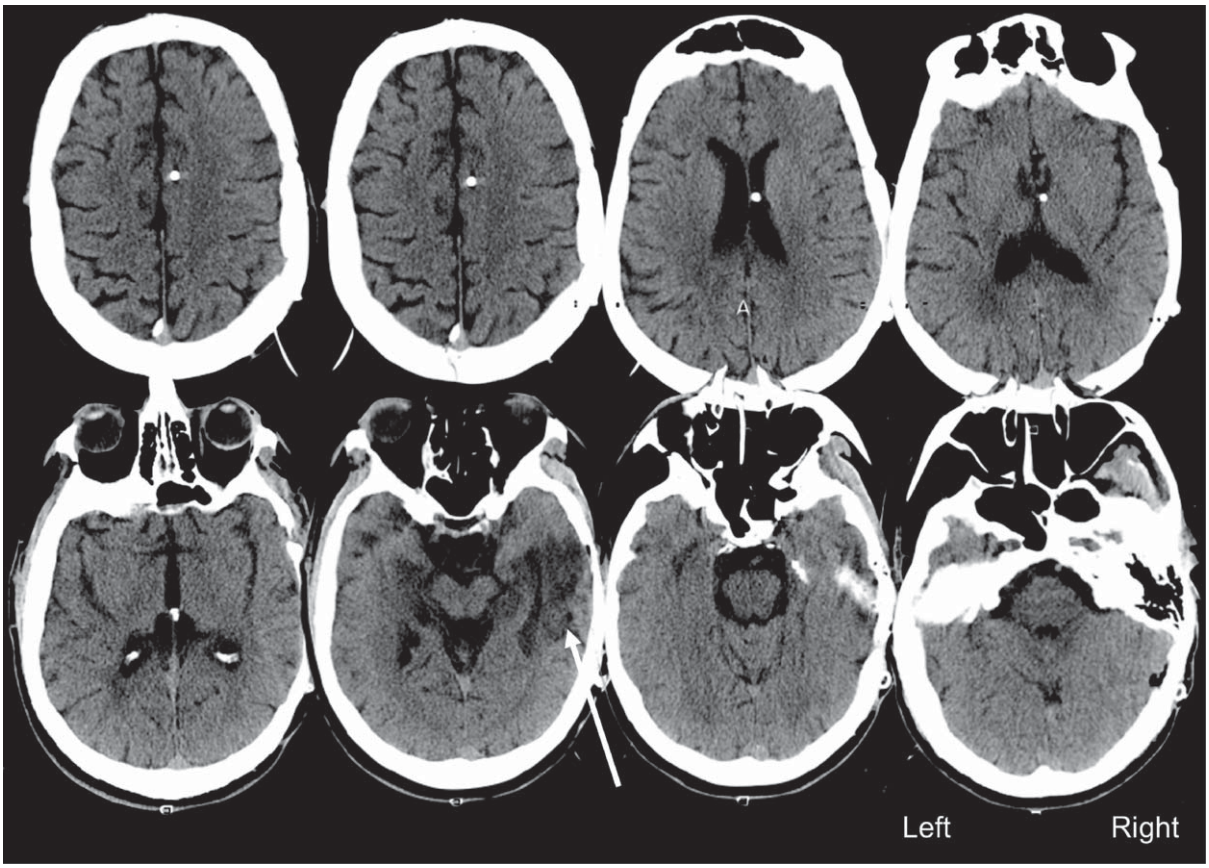


Fig. 1. The CT scan performed right before the midazolam-related "awakening" is presented. As apparent from the scan, the hypodense area roughly involves a part of the right temporal lobe.

midazolam instead of the usual propofol. Surprisingly SV began to interact with the anesthetist and soon after with his parents. He talked by cellphone with his aunt and congratulated his brother when he was informed of his graduation; he recognized the road leading to his home. When he was asked about his car accident he did not remember anything and apparently he was not aware of his status. This clinical status lasted about two hours after drugs administration and disappeared quickly after, taking the patient back to the previous clinical status (psicomotor slowness and lack of verbal initiative).

His relatives reported that SV did not show either word production or responsive behaviour during the time window following the CT scan.

SV behavioural examination was repeated one week and four weeks later. During both examinations we were able to observe only a single episode in which SV reacted to a simple command with a longer latency (i.e. "switch off the light" while he was walking out of the room). No further verbal commands were executed during these examinations.

To verify if SV clinical status modification had been induced by midazolam, we repeated the administration of midazolam (5 mg in 100 cc of saline solution in eight min), under both behavioural and EEG monitoring. The study was approved by the Pisa Hospital ethical committee and informed consent was obtained from the legal surrogates of SV.

Few minutes after midazolam administration, the patient began to interact with his brother and with the staff, answering appropriately to the questions that were asked. For this striking response to benzodiazepine administration we suspect, in our patient, the coexistence of catatonia.

Behavioural reactions preceding and following midazolam administration have been described and videotaped (watch movies: http://youtu.be/XIsTC4l_bHo and http://youtu.be/-rWzNv3q8Xc). EEG recorded before and after midazolam administration has been submitted to spectral power analysis, sLORETA analysis and intrinsic connectivity analysis within both the task-positive network and the linguistic network. Our attention has focused on these two particular networks because we believe that their functional improvement has substantially contributed to determine the awakening reaction presented by our patient. The task-positive network, in fact, has to deal with the ability to cope with and solve cognitive tasks that require explicit behavioural responses, while the linguistic one deals with language comprehension and production. All of these functions have been proven to recover following midazolam administration: our patient, in fact, was able to understand verbal commands and to perform any type of response was required, either behavioural or verbal.

2. Background

The incidence of traumatic brain injury is high both in industrialized and non-industrialized countries and it is estimated to be between 150–250 cases per 100.000 population per year (Thurman and Guerrero, 1999; Tagliaferri et al., 2006), about 10% of these can be classified at the admittance as severe TBI, based on the Glasgow Coma Score (GCS, <8). In the case of survival, after the phase of coma, the patient evolves either towards vegetative state, minimally conscious state, or severe disability, that result in catastrophic changes for his/her family and in a significant amount of human and financial resources from the national health and social systems.

In order to try to achieve the recovery of consciousness, either stimulant or depressant pharmacologic agents have been used in small-scale pharmacological trials.

As regard stimulant pharmacologic agents, studies using amantadine, a mixed of NMDA antagonist and dopaminergic agonist, showed a better outcome in post-traumatic disorders of consciousness (Whyte et al., 2005; Sawyer et al., 2008). Also the administration of other dopaminergic agents, such as levodopa and bromocriptine, appears to promote a favourable functional outcome (Passler and Riggs 2001; Matsuda et al., 2005).

Several reports concern the arousing effect of depressant pharmacological agents. Zolpidem, a selective non-benzodiazepinic GABAA alfa 1 agonist, has been extensively studied both clinically (Clauss et al., 2000, 2001; Shames and Ring, 2008; Nyakale et al., 2010), and with functional neuroimaging (Clauss and Nel, 2004; Brefel-Courbon et al., 2007; Victor et al., 2011).

Regarding other GABAergic drugs, just few case reports of patients with severe brain injury who have a significant clinical improvement after diazepam (GABAA agonist) (Caradoc-Davis, 1996) or baclofen (GABAB agonist) (Taira and Hori, 2007) administration are documented in literature.

3. Materials and methods

3.1. Behavioural evaluation

A procedure similar to that used for Wada Test was executed (Jones Gotman, 1987). Speech testing includes: naming the subject own name, naming of objects, reading of single words. Language comprehension was estimate using a yes-no response to recognition of subject name choosing among 4 verbally presented alternatives, a modified version of token test, while he had to choose among 4 objects, the one written on the sketchpad that was positioned in front of him. Finally a readapted version of Mini Mental State Examination (Folstein et al., 1975) was administered while the patient was lying in bed both before and after drug administration.

3.2. EEG recording and pre-processing

EEG activity was monitored from ten minutes before midazolam administration to twenty minutes after the administration. EEG signals were acquired using a BQ132S EEG amplifier (BrainQuick System, Micromed, Treviso, Italy) and an electrode cap (Electro-Cap International, Inc., Eaton, Ohio 45320 USA) at 19 positions following the 10–20 International System. EEG signals were collected with a sampling rate of 256 Hz and band-pass filtered between 0.03 and 45 Hz, keeping electrodes impedance below 5 kΩ. Independent component analysis (ICA) was employed to detect and remove EEG artifacts (Makeig et al., 1996). EEG signals were then visually inspected and residual artifacted epochs were removed. In line with a previous paper from our group (Bonfiglio et al., 2013), surface potentials were referenced off-line (after ICA pruning), to an estimated infinity reference using the REST software (Qin et al., 2010). The infinity reference was chosen as this technique has been demonstrated to perform better than other commonly used referencing schemes when analyzing power spectra (Yao et al., 2005), ERP topographies (Yao et al., 2007), and connectivity (i.e. coherence measures) (Qin et al., 2010).

At the end of the artifacted epochs removal procedure, about 5 minutes of recording before midazolam administration, 10 minutes encompassing the infusion period and 18 after were retained. All three periods (pre-infusion, infusion and post-infusion) were divided in 8 seconds epochs.

3.3. EEG spectral analysis

For each EEG channel and each epoch, the power spectrum was estimated using the wavelet transform (Torrence and Compo, 1998) with a Morlet basis. The Morlet is a complex function defined as: $\psi_0(\eta) = \pi^{-1/4} e^{i\omega_0\eta} e^{-\eta^2/2}$(1), where $\eta = t/s$ is a non-dimensional time parameter (t being the time-parameter expressed in time samples and s the scale), and ω_0 is a non-dimensional frequency that identifies the number of wavelet cycles (a value of 6 was chosen for the non-dimensional frequency to satisfy the admissibility condition, see for example Farge, 1992). The non-dimensional frequency was kept constant at different scales.

In the continuous limit the Fourier Transform (FT) of the function $\psi_0(t/s)$ is given by $\Psi_0(s\omega)$ (here and in the following upper-case letters indicate the FT of a function). The FT of the Morlet function is thus defined as: $\Psi_0(s\omega) = \pi^{-1/4} H(\omega) e^{-(s\omega-\omega_0)^2/2}$ (2), where $H(\omega)$ is the Heaviside step function ($H(\omega) = 1$ if $\omega > 0$, $H(\omega) = 0$ if $\omega < 0$). As the Morlet is a non-orthogonal wavelet basis, the wavelet function at each scale has to be normalized to have unit energy (so that transforms of different time-series and at different scales can be compared): $\Psi(s\omega_k) = (2\pi\delta t/s)^{1/2} \Psi_0(s\omega_k)$ (3), where k is the angular frequency index.

Let then x_n be the time series related to one epoch where $n = 0, 2, \ldots N-1$, N being the number of samples in the time series (2048, that is $8^* f_s$ where f_s is the EEG sampling rate) and $\delta t = 1/f_s$ be the time spacing between consecutive samples.

The Wavelet Transform (WT) of the x_n time-series at a scale s and time-point n is defined as the convolution of x_n with a scaled and translated version of the mother wavelet ψ:

$$W_n(s) = \sum_{\mu=0}^{N-1} x_\mu \psi * \left[\frac{(\mu - n)\,\delta t}{s}\right] \quad (4)$$

($\psi *$ indicates the complex coniugate of ψ)

While the wavelet transform can be estimated using Equation (4), a faster approach to the transform is to conduct the calculation in Fourier space. It is worth underlining that the approximation of the WT is obtained applying the above-defined convolution N times for each scale. All the N convolutions at a given scale can be conducted simultaneously in Fourier space using the Discrete Fourier Transform (DFT).

Let $X_k = \frac{1}{N}\sum_{n=0}^{N-1} x_n e^{-2\pi ikn/N}$ (5) be the DFT of x_n where k is the frequency index. The application of the convolution theorem leads to the following formulation: $W_n(s) = \sum_{k=0}^{N-1} X_k \Psi * (s\omega_k)\, e^{i\omega_k n\delta t}$ (7) where the angular frequency is $\omega_k = \frac{2\pi k}{N\delta t}$ for $k < N/2$ and $\omega_k = -\frac{2\pi k}{N\delta t}$ for $k \geq N/2$.

Finally the wavelet power spectrum expressed in dB is defined as: $P_{dB}(s) = 10\log_{10}\left(|W_n(s)|^2\right)$ (8) for each scale s, where $|W_n(s)|^2$ is expressed in μV^2.

For a wavelet analysis based on a non-orthogonal basis, an arbitrary set of scales can be used. The scales were expressed as fractional powers of two: $s_j = s_0 2^{j\delta j}$ (see Torrence and Compo, 1998) where the j were chosen in order to cover the scales (and corresponding frequencies) of interest; we set $s_0 = 2\delta t$ (i.e. 0.0078) in order to satisfy the condition on the equivalent Fourier period T (which should be at least equal to $2\delta t$, as for the Morlet function the following equation holds: $T = \frac{4\pi s}{\omega_0 + \sqrt{2+\omega_0^2}} \cong 1.03s$ (9) with the choice of $\omega_0 = 6$. Regarding δj, a value of about 0.5 is the largest one that gives adequate sampling in scale (Torrence and Compo, 1998); to obtain a finer scale resolution δj was set equal to 0.1. The frequencies of interest ranging from 1 Hz to 35 Hz were thus covered by 51 scales. Frequencies were obtained from scales applying the following $f_j = \frac{1}{1.03 s_j}$ which directly derives from (9). Equation (8) can be rewritten as a function of frequency as: $P_{dB}\left(f_j\right) = 10\log_{10}\left(\left|W_n\left(f_j\right)\right|^2\right)$. The mean power spectra of each epoch was then estimated for each channel. Power spectra (as a function of frequency) were obtained for each channel averaging among mean spectra of epochs pertaining to each period (be it pre- or post-infusion). The mean scalp power spectra as a function of frequency (see Fig. 2 central graph) was then obtained averaging among electrodes.

The time-course (from pre-infusion to post-infusion and including also the intra-infusion epochs) of mean scalp power was then estimated for seven EEG bands: delta (1–4 Hz), theta1 (4–6 Hz), theta2 (6–8 Hz), alpha1 (8–10 Hz), alpha2 (10–12 Hz), beta1 (12–18 Hz), beta2(18–35 Hz), see Fig. 3:

- For each channel and each 8 seconds epoch the power of each selected band was estimated as the summation of power values related to the center frequencies included in the band: a) delta (1.04–3.87 Hz); b) theta1 (4.15–5.87 Hz); c) theta2 (6.29–7.74 Hz); d) alpha1 (8.30–9.53 Hz); e) alpha2 (10.22–11.74 Hz); f) beta1 (12.58–17.79 Hz); g) beta2 (19.07–33.20 Hz).
- As a second step, the mean scalp power for each 8 seconds epoch and each band of interest was estimated averaging between electrodes.
- Finally, for each band and epoch, the power Z-score was estimated referred to the pre-infusion period: $Z_{ij} = \frac{P_{ij} - \langle P_{ik}\rangle}{std(P_{ik})}$ where i is the band (delta, theta1, theta2, alpha1, alpha2, beta1, beta2), j is the 8 seconds epoch (ranging from the beginning of pre-infusion to the end of post-infusion), k are the 8 seconds epochs included in the pre-infusion period, $\langle P_{ik}\rangle$ identifies the mean power related to the i-th band in the pre-infusion period (obtained averaging among epochs pertaining to the pre-infusion) and $std(P_{ik})$ identifies the standard deviation of power related to the i-th band during the pre-infusion period (estimated over the 8 seconds epochs of the pre-infusion).

3.4. EEG-current source localization analysis

Intracortical distributions of electrical activity in each band were estimated both for the pre-infusion and post-infusion epochs using standardized Low Resolution Tomography, sLORETA (Pascual-Marqui, 2002). sLORETA has been extensively used to localize sources of current with set-ups of as few as 19 electrodes (Clemens et al., 2010; Canuet et al., 2011).

Montreal Neurologic Institute average MRI brain (MNI152) (Mazziotta et al., 2001) was used as a realistic head model (6239 cortical gray matter voxels at 5 mm resolution) for which the lead field was computed. Electrode positions were registered to the spherical model on the basis of Towle et al. (1993). It is fair to underline that the use of standardized cortical structures instead of the real ones of the subjects (as obtained by MRI) inevitably leads to inaccuracies of source localizations. Valdés-Hernández et al. (2009), examined the performances of various standardized head models finding mean localization errors varying between 6 and 8 mm depending on the chosen head model.

On the other side, sLORETA has been proven able to reliably identify activations even in deep cortical structures such as the cingulate cortices confirming findings obtained with other brain imaging techniques such as PET (Pizzagalli et al., 2001) or fMRI (Olbrich et al., 2009).

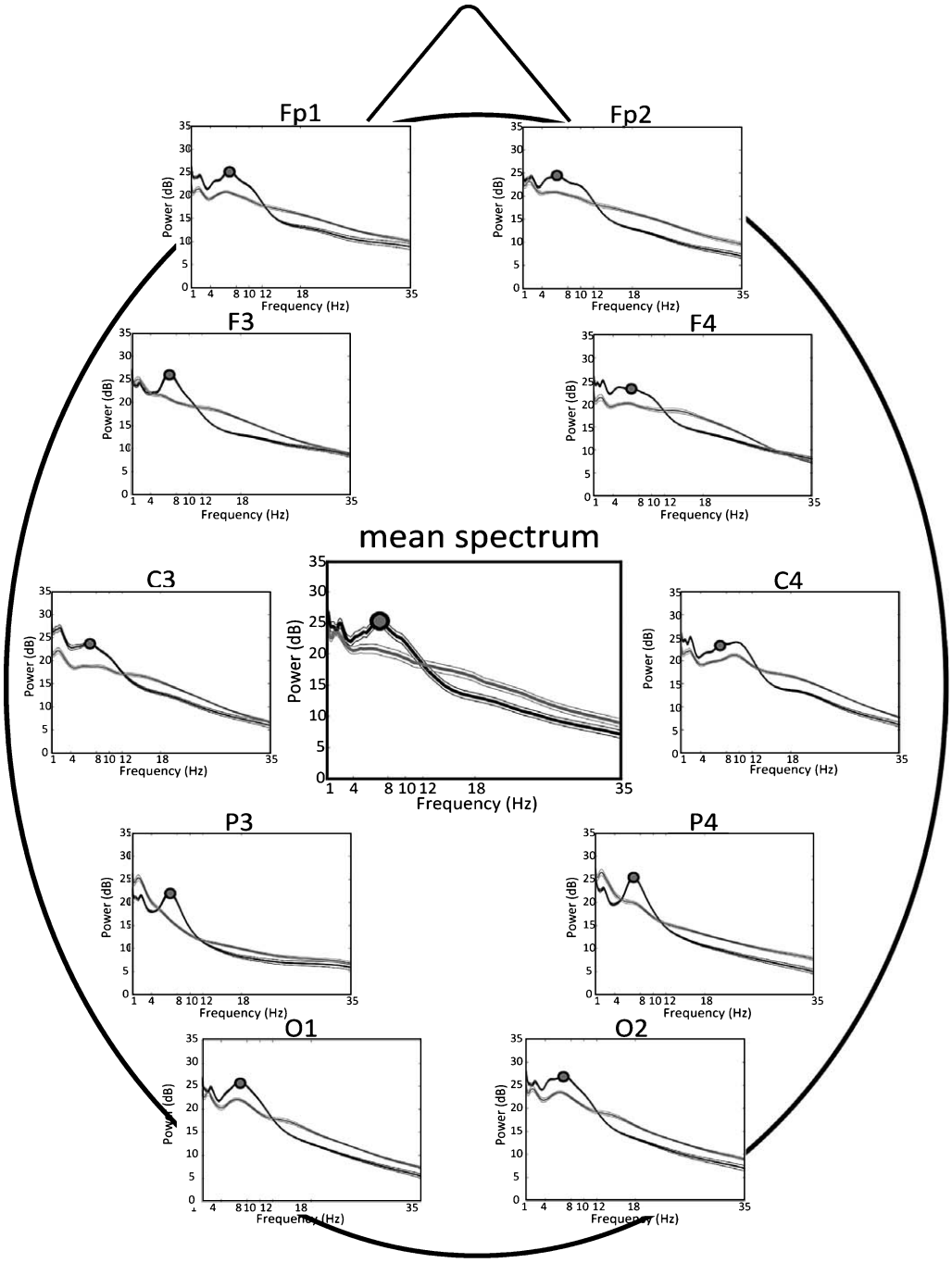


Fig. 2. Power spectra of pre-infusion (black lines) and post-infusion (grey lines) for ten selected electrodes are presented. For each electrode and condition (pre- or post-infusion) the mean power spectrum was obtained mediating between the power spectra of the 8 seconds epochs pertaining to the condition. The mean power spectra (averaged over all electrodes) of pre-infusion (black line) and post-infusion (grey line) are depicted in the graph at the center of the scalp. Power spectra are expressed in dB. For visualization purposes, as the frequencies are discrete, the continuous line depicting the power spectra as a function of frequency was obtained interpolating among the available frequencies. Note the presence of a peak at 7 Hz for the pre-infusion. Post-infusion is characterized by an enhancement of higher frequency activity (>12 Hz) and a parallel decrease of lower frequencies (<12 Hz).

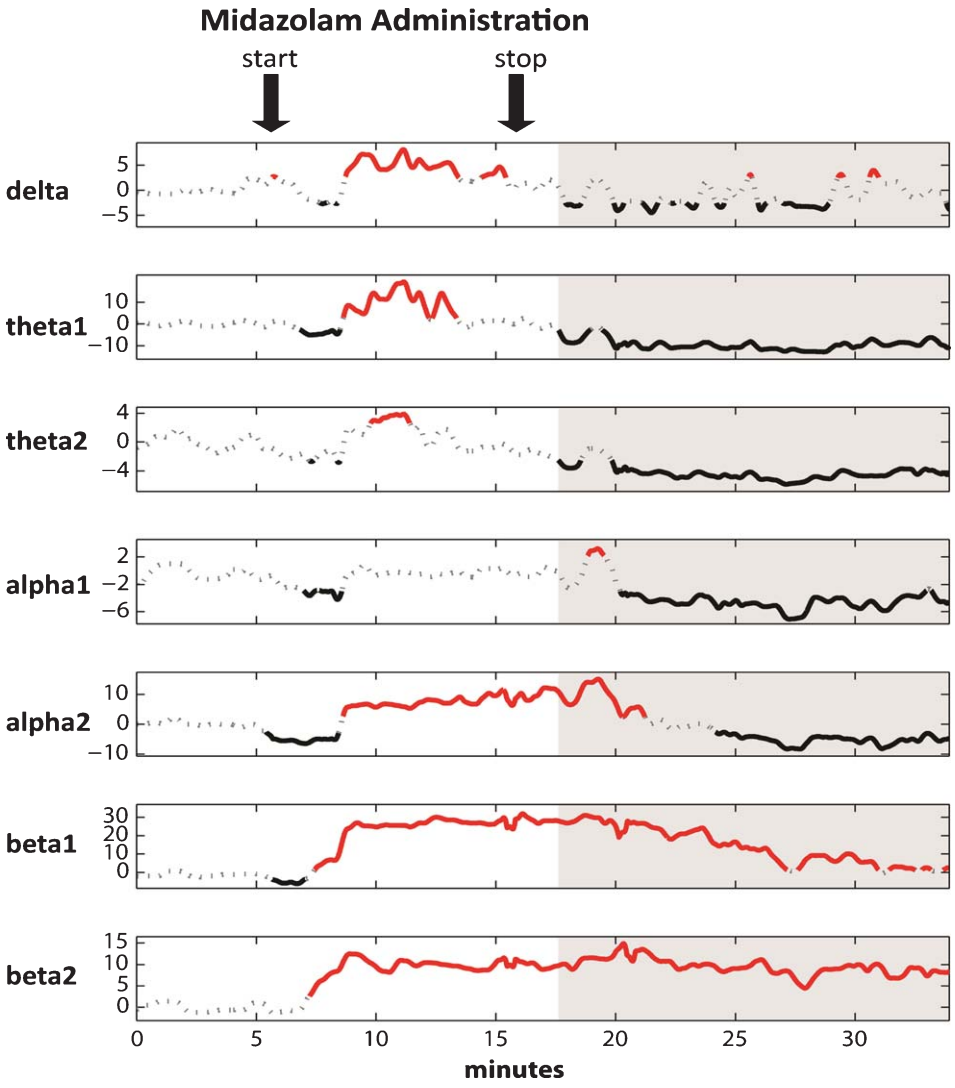


Fig. 3. Z-scores of the time courses of seven frequency bands are presented. The time course of each band was obtained averaging between the time courses of single electrodes. The Z-score of each 8 seconds epoch was estimated related to the 8 seconds epochs included in the pre-infusion period. For each band, the time-course is represented by a gray dotted-line for epochs whose Z-scores are lower than the significance threshold ($Z = 2.58$ in absolute value, corresponding to $p < 0.01$). The time-course is represented by a solid black line for epochs whose Z-score is lower than -2.58 and by a solid red line for epochs whose Z-score is higher than 2.58. Two-three minutes after the beginning of midazolam infusion a broadband increase of power is observed. After the end of midazolam administration a significant decrease of theta1, theta2, alpha1 and alpha2 takes place, whereas beta1 and particularly beta2 still yield values significantly higher than those of the pre-infusion. In each subplot the period during which the patient regained full consciousness is shaded in grey.

Significant differences in sources distributions between pre and post-infusion epochs were assessed for each band on the basis of *t*-tests with a Statistical Non-Parametrical Mapping (SNPM, Nichols and Holmes, 2002) approach for multiple testing. In order to take into account the simultaneous testing on multiple voxels, a single threshold test approach (i.e. a single critical *t*-value derived from 10000 permutation performed for each voxel in each band was estimated) was used.

SNPM works as follows: let us consider the null hypothesis of no significant difference between epochs pertaining to each condition (be it pre- or post-infusion): under the null-hypothesis, the labeling of

epochs for each period could be changed randomly (i.e. the value related to an epoch of the pre-infusion could be assigned to post-infusion and vice-versa). Based on this assumption, 10000 random relabeling were made and for each of them, the *t*-value related to each voxel was estimated. For each relabeling, only the maximum t-value (in absolute value) among voxels was retained, obtaining thus the maximum statistics distribution under the null-hypothesis. For each band, the significance of the *t*-value related to each voxel was estimated as the ratio between the number of t-values of the distribution obtained under the null-hypothesis exceeding the original *t*-value (in absolute value) and the number of relabeling. The *t*-value threshold (in absolute value) corresponding to $p < 0.0014$ (which leads to an overall $p < 0.01$, applying Bonferroni correction, as the tests were conducted on multiple bands), was estimated for each band, and the maximum t-value threshold among the bands was taken. As already mentioned above, the issue of multiple testing on different EEG bands was dealt with applying Bonferroni correction for multiple comparisons. The overall significance threshold was set at $p < 0.01$, which corresponds to a threshold of $p < 0.0014$ for each band.

3.5. *Phase synchronization analysis in the task positive network and in the semantic network*

Connectivity between regions of interest were estimated for each 8 seconds epoch both during the pre-infusion and the post-infusion on the basis of phase synchronization.

Let $x(t)$ and $y(t)$ be two time-series, the squared phase synchronization is defined as:

$$\varphi^2_{X,Y}(\omega) = \left| \frac{1}{N_r} \sum_{j=1}^{N_r} \breve{X}_{j\omega} \breve{Y}^*_{j\omega} \right|^2$$

where $\breve{X}_{j\omega}$ and $\breve{Y}_{j\omega}$ indicate the normalized Discrete Fourier Transform of respectively $x(t)$ and $y(t)$; Nr is the number of segments on which the Fourier Transform is being applied. Normalization is obtained as follows:

$$\breve{X}_{j\omega} = \left(X^*_{j\omega} X_{j\omega} \right)^{-\frac{1}{2}} X_{j\omega}$$

(see Pascual−Marqui, 2007).

Phase synchronization was evaluated in each epoch for each frequency band considering two cortical networks: the Task Positive Network (TPN) and the Semantic Network (SN). Regions included in the TPN were chosen in line with (Fox et al., 2005), whereas Semantic Network regions were taken from Binder et al. (2009) (see Table 1 for details). For the analysis of connectivity a single voxel at the centroid of each region was used. This procedure is justified because sLORETA has a relatively low spatial resolution which makes it unable to separate two closely spaced sources, and additionally, the centroid voxel is an excellent representative of the corresponding region (Canuet et al., 2011). For each network, phase synchronization was estimated between all couples of regions, both for the pre-infusion and the post-infusion periods.

Table 1
For each of the two considered network, regions and region centroids in MNI coordinates are presented

Network	Cortical structure	Hemisphere	Centroid (MNI coordinates, x,y,z in mm)
TPN	Frontal Eye Field, FEF	Left	(–25, –15,55)
		Right	(25, –15,55)
	Intraparietal Sulcus, IPS	Left	(–25, –60,45)
		Right	(25, –60, 45)
	Middle Temporal Region, MT	Left	(–45, –70, –5)
		Right	(45, –70, –5)
SEMANTIC NETWORK	Inferior Frontal Gyrus, IFG	Left	(–40, 22, 0)
		Right	(41, 22, 1)
	Medial Prefrontal Cortex, MPFC		(0, 50, 0)
	Middle Temporal Gyrus, MT	Left	(–54, –37, –6)
		Right	(55, –33, –8)
	Parahippocampal and Fusiform	Left	(–32, –41, –19)
	Gyri, PHF	Right	(33, –41, –18)
	Posterior Cingulate, PC		(0, –57, 13)
	Posterior Inferior Parietal	Left	(–49, –44, 42)
	Lobe, IPL	Right	(49, –45, 43)

Significant differences in connectivity between post and pre-infusion were assessed separately for the two networks on the basis of *t*-tests with the same SNPM approach used for sLORETA. Also in this case, as the statistical tests were conducted on multiple couples of regions, for each band of interest a single threshold approach was used, with the aim of controlling for type I errors (Nichols and Holmes, 2002). Correction for multiple bands testing was conducted applying Bonferroni method. The overall significance threshold was set at $p < 0.01$ (corresponding to a threshold of $p < 0.0014$ for each single band).

As the choice of the epoch length (8 seconds) is arbitrary, both the analyses on current sources distributions and on phase synchronizations, were repeated using epoch lengths varying between 4 seconds and 12 seconds with 1 second steps, in order to exclude possible dependencies of our findings on the specific epoching applied (i.e. the same analyses were repeated considering epochs of 4, 5, 6 up to 12 seconds). Both for sLORETA and phase synchronization only results (i.e. voxels for sLORETA, connectivity levels between couples of regions for phase synchronization) that were significant for all the epoch lengths (ranging from 4 to 12 seconds) were considered significant (see Supplementary Material for results regarding 4, and 12 seconds epoching, results with other epoch lengths are not shown).

4. Results

4.1. Midazolam-induced behavioural changes

After midazolam infusion, SV became more cooperative and appeared as "just awakened". The speech production, although not perfectly articulated, was understandable. He was able to answer with simple sentences to specific questions. He could pronounce his own name and the name of simple objects brought to his sight. He could also read simple object nouns. He understood simple sentences such as "touch a yellow square". He was also able to read and understand the sentence "close your eyes". SV was not oriented either in time or in space except for the place where he was. SV was able to compute simple subtractions (i.e. 100–7, 93–7, etc.). He did recognize his relatives and he did recall the names of two of his grandchildren. He was even able to recognize the way to his home (as reported by his parents). He failed to remember objects presented before midazolam administration when they were presented again after the infusion.

4.2. Midazolam-induced EEG power spectrum changes

As it can be seen from Fig. 2 (black line), the EEG power spectrum at baseline (pre-infusion) is characterized by a) high power at low frequency (1–12 Hz), with a characteristic power peak in the theta2 band, (at about 7 Hz), which is detectable across different regions of the scalp and b) relatively low power in the frequency range between 12 and 30 Hz.

After midazolam infusion (post-infusion period), a change in the power spectrum profile is observed (Fig. 2, grey line): a) the power between 1 and 12 Hz is reduced, the highest decrease (with respect to the pre-infusion) at about 7 Hz (theta2 band) and b) the power between 12 and 30 Hz is increased, with a maximum difference when compared to pre-infusion at about 15 Hz (beta1 band). The end result is a more balanced distribution of the signal power across low and high frequencies, which results in an overall tendency towards a profile/spectral pattern normalization.

4.3. Time-course of the EEG power changes for each frequency band as a function of midazolam infusion and clinical modifications

During midazolam infusion, the most striking phenomenon was the power increase in the delta, theta1, alpha2, beta1, and beta2 bands (a consistent increase of both low and high frequencies), which started within the first 5 minutes after the infusion beginning to reach a plateau between the 5th and 10th minute (Fig. 3).

The power increase of alpha2, beta1, and beta2 bands, however, was more stable and lasted longer than that of delta and theta1 (Fig. 3). Within the first 5 minutes from the infusion end, in fact, high frequencies (beta1 and beta2) still remained at significantly higher levels with respect to the baseline, whilst low frequencies had already fallen to levels similar to the baseline (Fig. 3). Although in this period the low and high frequencies behaviour was already different, no clinical response was still observed. Clinical effects were observed more than 5 minutes after the beginning of the post-infusion period, when the low and high frequencies behaviour became totally dissociated, since lower frequencies (from delta to alpha1 bands) were reduced

to significantly lower levels with respect to the baseline while high frequencies (beta1 and beta2 bands) still remained at significantly higher levels (Fig. 3). It's worth noting the intermediate behaviour of the alpha2 band, which increased along with high frequencies in the first 5 minutes after the infusion, but decreased together with low frequencies in the next few minutes (Fig. 3).

4.4. Cortical source localization of the midazolam-induced changes for each frequency band

By inspecting Fig. 4, consider that the pre-infusion period was taken as a reference and, as such, treated as a baseline. Consequently, cold colors (blue tones) represent cortical areas in negative variation, that is where the current density levels decreases significantly after midazolam infusion, and warm colors (yellow, orange) those in positive change, that is where the current density increases significantly after the infusion. Keeping in mind this reading key, it becomes clear that midazolam significantly reduces lower frequencies (delta, theta1, theta2, alpha1, and alpha2 bands) activity on anterior cortical regions and, at the same time, boosts higher frequencies (beta1 and beta2 bands) activity on posterior cortical regions.

Let us now consider in detail each frequency band. The delta band regression is topographically limited to the lesion area and neighboring areas (the right fronto-temporo-insular and temporo-polar regions) (Figs. 2 and 4).

The theta band regression concerns, however, not only the lesion area, but also broad regions outside the lesion area, both in the ipsilateral (especially the dorso-lateral, latero-ventral and supraorbital prefrontal regions) and contralateral hemisphere (centro-parietal, frontal and prefrontal regions) (Fig. 4). This is particularly true for the theta2 band (peak at 7 Hz), which shows an even more marked regression on the contralateral than on the ipsilateral hemisphere (Fig. 4). A massive regression of both theta1 and theta2 bands also occurs on bilateral mesial prefrontal regions (mesial orbitofrontal and anterior cingulate) (Fig. 4).

The alpha band has lower activations (in the post-infusion) in juxta-lesional areas of the frontal, prefrontal and centro-parietal regions (Fig. 4). Compared to alpha2, the alpha1 band regression is more pronounced on the hemisphere contralateral to the lesion (pre-frontal and temporo-polar regions) (Fig. 4).

The beta band, finally, is the only one with an enhanced activity after the infusion. It increases, particularly, on the posterior regions, both mesial (precuneus, posterior cingulate, cuneus, and inferior temporo-occipital) and dorso-lateral (parieto-occipital) (Fig. 4). Compared to beta2, the beta1 band is characterized by the expansion on the left temporo-parietal junction (angular and supramarginal gyri), bilateral mesial pre-frontal cortex (anterior cingulate) and fronto-temporo-insular lesion area (Fig. 4).

4.5. Midazolam-induced changes within the task-positive network and the linguistic network cortical connectivities for each frequency band

We tested the task-positive network and the linguistic network because we believe such systems represent the functional basis of the individual interactivity with the outside world and people.

The baseline condition is characterized by a higher intrinsic connectivity of these systems in the low frequencies (Fig. 5), which, probably, corresponds to the pre-infusion prevalence of such frequencies both in terms of power spectrum and current densities distribution (cortical sources). This occurs for both networks, but for the linguistic one to a greater extent. Among all the bands, the one that seems to affect the intrinsic connectivity of this circuit to a greater extent is the theta2 (peak at 7 Hz). It is as if the intrinsic connectivity in both systems was saturated by oscillations at about 7 Hz and thus not accessible to other frequencies.

Conversely, the midazolam infusion appears to remove the low frequency intrinsic connectivity (in particular in the theta2 band) and to foster it in the high frequencies (in particular beta1 band) in both networks, both at the intra-hemispheric or inter-hemispheric level (Fig. 5).

5. Discussion

To our best knowledge, this is the first case described in literature in which a patient with a clinical diagnosis of MCS has shown a paradoxical behavioural facilitation in response to the administration of midazolam (a non-selective GABA A agonist of the benzodiazepine class). On the other side, although relatively rare (1 out of 15 consecutive cases, according to Whyte and Myers, 2009), the response to zolpidem (an alpha1-selective GABA A agonist of the imidazopyri-

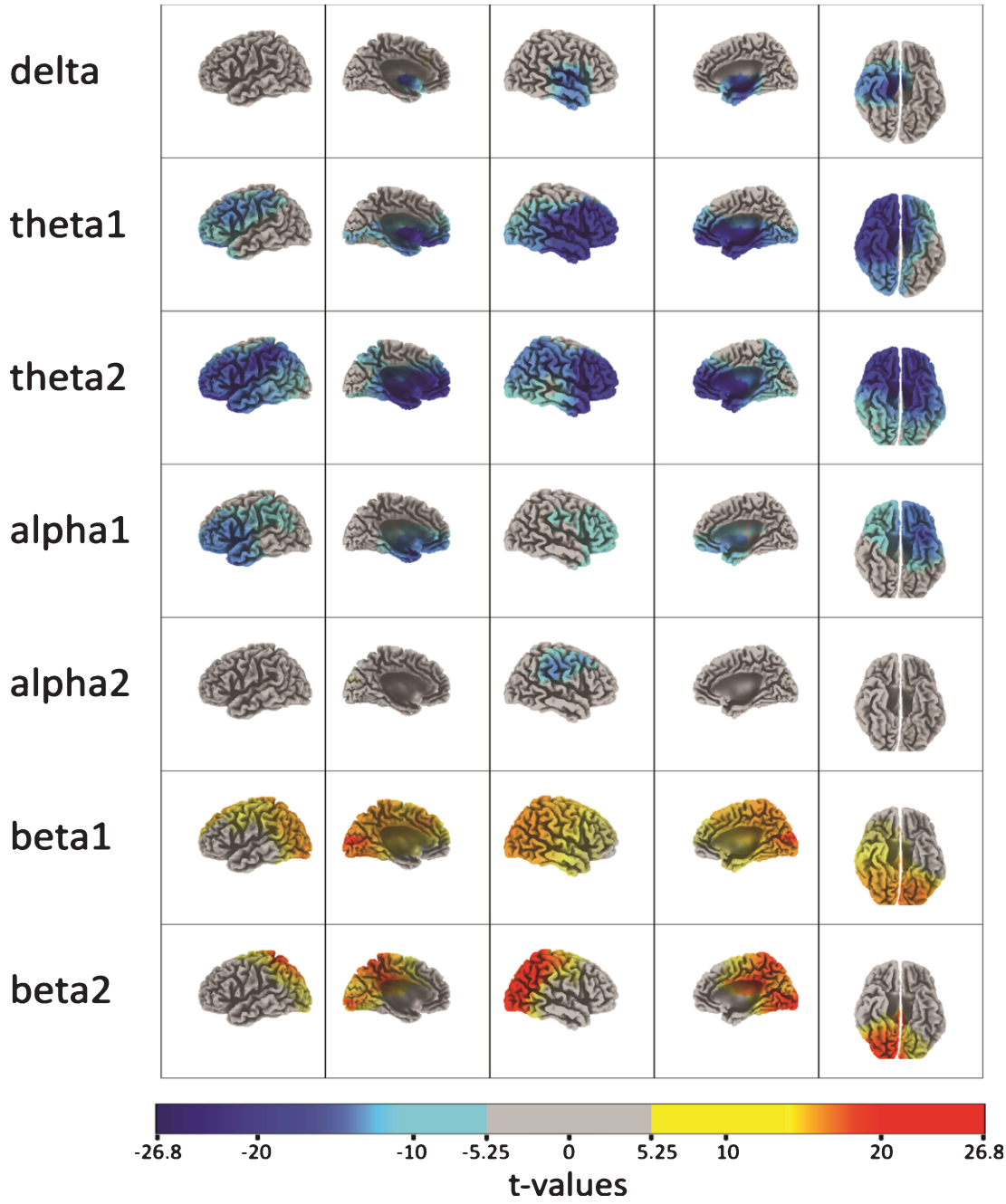


Fig. 4. Voxels with significantly different levels of activation between post and pre-infusion, ($|t| > t_{crit}$, overall $p < 0.01$, $t_{crit} = 5.25$) are presented for each of the 7 bands. Panels refer to delta, theta1, theta, alpha1, alpha2, beta1 and beta2 respectively. Yellow to red tones correspond to progressively higher (i.e. with higher *t*-values) activations in the post-infusion whereas light to dark blue tones indicate a progressively higher activation in the pre-infusion. First column of each panel represents a left view of the left hemisphere, second column a right view of the left hemisphere, third column a right view of the right hemisphere, fourth column a left view of the right hemisphere and fifth column a view from below of the whole cortex.

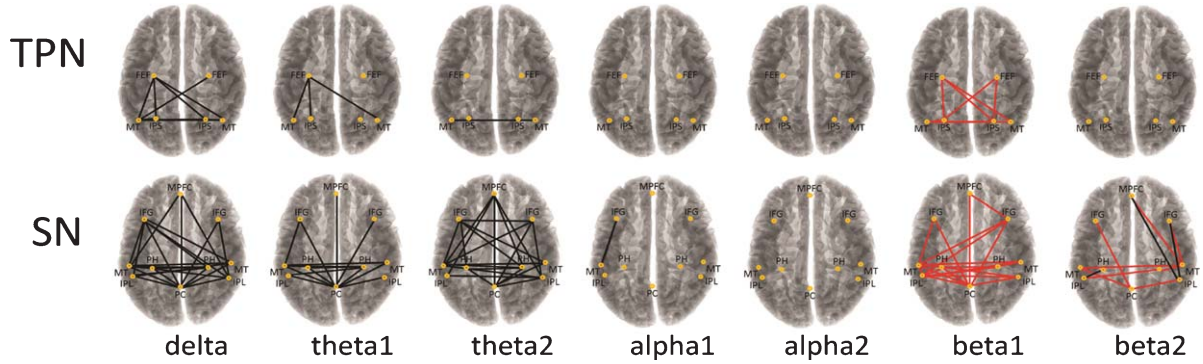


Fig. 5. Significant differences of phase synchronization are presented for two networks of interest (Task Positive Network, TPN, and Semantic Network, SN). Black tones indicate significantly higher phase synchronization during the pre-infusion (overall $p < 0.01$) whereas red tones indicate significantly higher levels during the post-infusion (overall $p < 0.01$).

dine class) is well documented in literature (Clauss et al., 2000, 2001). It should be emphasized that our patient, despite the attempts made, was not responsive to zolpidem.

The EEG quantitative analysis in basal conditions showed an abnormal power peak at about 7 Hz spread all over the scalp with a high intra- and inter-hemispheric coherence (hyper-synchronous theta) within both the task-positive and the linguistic functional networks. These EEG abnormalities together with the behavioural unresponsiveness state, critically and temporarily recovered after midazolam administration.

Thus, the case presented here seems to contradict to some extent what has recently reported by Williams and colleagues (Williams et al., 2013) about the fact that the 7 Hz peak may be a predictive sign about patients' responsiveness to the specific administration of zolpidem. On the contrary, in the light of the findings herein described, the presence of the 7 Hz peak could indicate a possible responsiveness towards GABA A agonists "latu senso", whether selective (such as imidazopyridines) or non-selective (such as benzodiazepines).

In addition, the analysis of midazolam-induced EEG changes in the different domains of frequency, time and topography, along with the study of the intrinsic connectivity of two functional strategic networks, has led us to a) formulate some hypotheses about certain aspects of the recovery from the MCS both in terms of anatomo-functional correlations and system neuroscience and b) make inferences about the role that some kind of 'catatonic' symptoms might play in determining and/or maintaining this peculiar clinical state.

5.1. Frequency-domain

As clearly observable in Fig. 2, our patient's EEG power spectrum presents evident elements of abnormality, showing an high power between 1 and 12 Hz and relatively low power between 12 and 30 Hz. This pattern, characteristically biased towards low frequencies at the expense of the higher ones, is in line with what has been widely reported in literature regarding subjects with DOC (Lehembre et al., 2012). Moreover, the presence of a well-defined power peak at about 7 Hz which is widely distributed all over the scalp and is very similar to the recently reported one in the baseline EEG of three zolpidem-sensitive patients with DOC (Williams et al., 2013), clearly emerges.

After midazolam infusion a significant change of the power spectrum profile (Fig. 2, grey line) is observed: a) the power level in the frequency range between 1 and 12 Hz is reduced, with the marked reduction or, for most of the electrodes, the disappearance of the 7 Hz peak and b) the power in the frequency range between 12 and 30 Hz increases, with its maximum effect for the beta1 band. Both the basal (pre-infusion) EEG pattern (theta2 and beta1) and the midazolam-induced (theta reduction and beta increase) anti-correlated changes showed by our patient are similar to those reported by Williams and colleagues (2013) in their zolpidem-responders patients and are compatible with the cellular mechanism proposed by these authors to

motivate the origin of the 7 Hz peak and its high and widespread coherence. The 7 Hz rhythm would be in fact the product of "self-sustained intrinsic membranes oscillations arising from Layer V pyramidal neurons" under the influence of a global thalamic suppression and/or disfacilitation due, in turn, to a deafferentation produced by a severe and widespread cortical damage.

In contrast, our patient had a right focal fronto-temporo-opercular cortical lesion (Fig. 1, CT-scan). Given this assumption, according to the model proposed by Williams and colleagues (2013), we would have to expect rather the typical EEG pattern of a thalamo-cortical dysrhythmia, i.e. a focal and congruent increase of both theta and beta oscillations under basal conditions and an equally congruent reduction in response to pharmacotherapy. None of this, however, was observed in our case.

5.2. *Time-domain*

During the midazolam infusion, the most striking phenomenon consisted in an initial congruent/consistent increase of both low and high frequencies (delta, theta1, alpha2, beta1, beta2 bands) power which reached a plateau shortly before the infusion end (Fig. 3). In the first 5 minutes after the end of the infusion, low frequencies did already fall to levels similar to the baseline, while the higher ones (beta1 and 2) remained at significantly higher levels (Fig. 3). Although in this period the behaviour of low and high frequencies was already different from baseline, no clinical response was observed. A clinically significant response was apparent more than 5 minutes after the infusion, when the behaviour of lower and higher frequencies became openly anti-correlated, as the lower frequencies (delta to alpha1) were reduced to significantly lower levels with respect to the baseline, while the high ones (beta1 and beta2) still held at significantly higher levels (Fig. 3).

The ability to increase the high frequencies intensity is a known property of the GABA A agonists (so-called paradoxical effect), either of those belonging to the benzodiazepine class or to the imidazopyridine class. However, taking into account only the time-domain, the decisive event for the clinical improvement appears to be the abatement of low frequencies below basal levels, although a possible permissive role of the high frequencies increase cannot be ruled out.

A tentative hypothesis could be that, since the clinical improvement coincides with the temporal coexistence of both variations, it occurs at the attainment of a certain critical value (threshold value) of the power ratio between these two frequency bands.

5.3. *Space-domain*

An overview of the Fig. 4, allows one to immediately realize that midazolam administration significantly reduces low frequencies (delta, theta1 and 2, alpha 1 and 2 bands) current densities on the anterior regions and that, at the same time, it boosts the higher ones (beta1 and beta2 bands) on the posterior regions. The most striking aspects, however, specifically concern beta and theta bands.

On the basis of the observations made in the power spectrum paragraph, we can argue that the regions where low frequencies are decreased after the infusion correspond to areas where these frequencies were abnormally increased before the infusion itself, as well as those where high frequencies are increased after infusion correspond to areas where they were previously poorly represented. In other words, we can say that the *blue* of the Fig. 4 represents the areas where low frequencies 'give ground' after the infusion, and the *red* those areas where the higher ones 'gain ground'. One can, therefore, consider the 'territorial dominance' of low frequencies at the expense of the high ones typical of the baseline, as the sign of the dysfunctional phenomenon responsible for the clinical syndrome. On the contrary, the post-midazolam clinical improvement, together with the related recovery of the bioelectric activity resides both in a 'retreat' of low frequencies and in an 'advancement' of the higher ones, which are often co-localized.

The theta band regression, in particular, involves not only the lesional area, but also wide extra-lesional regions, both of the ipsilateral hemisphere (especially dorsolateral prefrontal, ventral and lateral supraorbital regions) and of the contralateral one (central-parietal, frontal and prefrontal regions) (Fig. 4). This is particularly evident for theta2, which shows a more marked regression on the contralateral than on the ipsilateral hemisphere (Fig. 4). This probably results from an abnormal representation of theta oscillations on large bi-hemispheric cortical regions (suppressed areas) prior to the infusion, as a sign of either diaschisis (Andrews, 1991; Juhasz, 1997) or inhibition/disfacilitation phenomena of subcortical nature (e.g., thalamic) with a diffuse cortical expression (Hindriks and van Putten, 2013). A massive regression

of both theta1 and theta 2 bands also occurs upon bilateral mesial prefrontal regions (mesial orbitofrontal and anterior cingulate regions) (Fig. 4).

Finally, the beta band is the only one with higher current densities after the infusion. It increases, in particular, upon the posterior regions, both on the mesial (precuneus, posterior cingulate, cuneus, inferior temporo-occipital) and dorso-lateral (parieto-occipital) areas (Fig. 4). Compared to beta2, beta1 is characterized by its expansion on: left temporo-parietal junction (angular and supramarginal gyri), bilateral mesial prefrontal cortex (anterior cingulate cortex) and right temporo-insular lesional area. This, among other things, enables the increase of the inter-hemispheric coherence between homologous crucial nodes within the language network, as will be seen in the next paragraph (Fig. 4).

Taking into account only the topographic-domain, regardless of the diachronic time factor, critical elements associated with the functional recovery would seem to be both a widespread reduction of the 7 Hz peak upon wide anterior regions, and a reconquest of strategically important 'bridgeheads' by beta1 oscillations.

In particular, the functional recovery of both a) precuneus/posterior cingulate, whose progressive reactivation has long been recognized as characteristic of the recovery path from Vegetative State/Unresponsive Wakefulness Syndrome (VS/UWS) to normal (Laureys et al., 1999, 2006; Vanhaudenhuyse et al., 2010; Bonfiglio et al., 2013), and b) left temporo-parietal junction, recently referred to as the prerogative of those MCS patients who begin to follow commands (i.e., those who evolve from condition MCS− to MCS+) (Bruno et al., 2012; Bonfiglio et al., 2014), appear relevant. Moreover, in view of a possible diagnosis of catatonia (as will be discussed below), the bilateral reactivation of the mesial prefrontal region (mesial orbitofrontal and anterior cingulate cortices) and of the supplementary motor area assumes a special importance. Recent studies have in fact shown that motor, behavioural and affective symptoms in catatonia are connected to an altered pattern of activation in those specific regions (Northoff et al., 2004; Scheuerecker et al., 2009).

5.4. *Linguistic and task-positive networks intrinsic connectivity*

As stated above, we tested the task-positive and linguistic networks because we believe that they are representative of many interaction interfaces of the individual with the outside world and people, respectively.

The baseline condition (pre-infusion) is characterized by a greater intrinsic connectivity of these systems in the delta, theta1 and theta2 bands (Fig. 5), as a plausible sign of their functional deficit. This is particularly evident for the semantic network, whose intrinsic connectivity is blocked by 7 Hz oscillations.

Conversely, the midazolam infusion removes the intrinsic connectivity at low frequencies and fosters it at higher ones in both networks (Fig. 5), most likely promoting both the functional recovery and the resumption of the interactive and communicative capabilities of our patient towards the external environment and people. This seems to be favoured by the widespread reduction of the 7 Hz peak upon extended anterior regions and by the reconquest by beta1 of some strategic areas, such as the homologous middle temporal gyrus (MT) and inferior parietal lobule (IPL) nodes (i.e., the angular and supramarginal gyri of both hemispheres) within the linguistic network.

5.5. *Mesocortical circuit and severe disorders of consciousness*

A deficit of the frontal/prefrontal cortico-striatopallidal thalamocortical loop system (mesocortical circuit, see Fig. 6) has been recently proposed

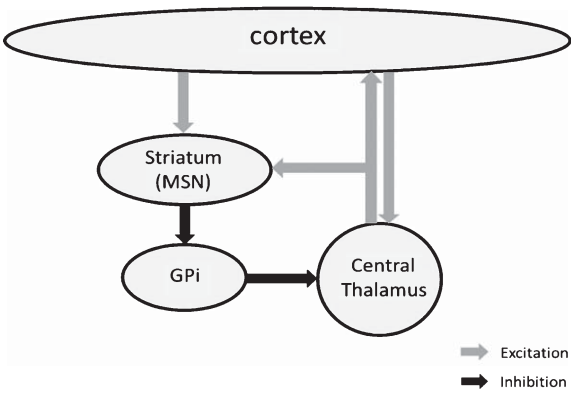


Fig. 6. This figure (modified from *Schiff, N.D. (2010). Recovery of consciousness after severe brain injury: The role of arousal regulation mechanisms and some speculation on the heart-brain interface. Clev Clin J Med, 77(Suppl 3), S27-S33*) represents the pathophysiological model (so-called "mesocircuit" model) proposed by Schiff to explain behavioural fluctuations following large-scale cortical dysfunction following multifocal brain injuries.

(Schiff, 2008, 2010; Williams et al., 2013) as the anatomical and patho-physiological basis of the severe disorders of consciousness resulting from diffuse axonal injury, anoxia and hypoxia-ischemia. A diffuse cortical damage resulting from these pathologies would lead to a neuronal cell loss and a widespread deafferentation of the central thalamus, either directly (cortico-thalamic subsystem) or indirectly (cortico-striato-pallidal subsystem). This would contribute to keep suppressed both wide bilateral regions of the frontal/prefrontal cortex (thalamo-cortical subsystem) and the striatum (thalamo-striatal subsystem). Both the striatum and the globus pallidus interna (GPi) would play a central role in this circuital mechanism. The former, under normal conditions, would exert its inhibitory activity towards the GPi, that is maintained by adequate cortical and thalamic inputs which ensure an adequate background activity of medium spiny neurons (MSNs) of the striatum. Lacking such an intense background activity, the GPi would be free to inhibit the central thalamus contributing to keep suppressed its activity. According to Schiff (2010), zolpidem's effectiveness in awakening patients with severe disorders of consciousness resides in the ability of this drug, highly selective for GABA A alpha 1 receptor, to maintain the inhibition of the GPi (which is characterized, in fact, by the highest expression of alpha 1 receptors), thereby releasing the pallidal inhibition of the central thalamus and restoring the functionality of the entire circuit.

The failure to respond to zolpidem observed in our patient, may be due to the great expression variability of GABA receptor subtypes detectable in the human population (currently at least 50 different phenotypes, according to Kang et al. (2011). So, in the specific case, the mesocortical mechanism could be simply mediated by different receptor subtypes. A patient with a receptor set-up characterized by a low expression of alpha 1 on sites with usually specific mediation (GPi and cortical inhibitory interneuronal networks), could see his/her mesocortical mechanism predominantly mediated by other receptors and as a consequence, to be less or not at all responsive to zolpidem but conversely, more or only responsive to midazolam. It is very interesting to underline that repetitive seizures and/or a status epilepticus are able to change the phenotypic representation of GABA A receptor subtypes, in particular by significantly reducing the expression of alpha1, as it has been recently demonstrated (Grabenstatter et al., 2012).

Alternatively, the response to midazolam observed in our patient, could be mediated through GABA A non-specific sites, which are present within the same loop, but in different locations than the GPi (for example, MSNs and pyramidal cell axons), obtaining in this way "the release of tonic inhibition of neurons from the globus pallidus interna" with an indirect rather than direct mechanism.

What still does not agree with the model proposed by Williams and colleagues (2013), is that, in our case, we do not have to deal with a diffuse neuronal damage, but with a focal damage.

5.6. Critical focal dysfunction, non-convulsive status epilepticus and catatonia

We propose here a hypothesis able to resolve the apparent discrepancy between the lesion focality and the widespread electrical abnormalities observed in our patient and, at the same time, to reconcile them with the positive response to midazolam and the lack of response to zolpidem.

Considering, in fact, the typical 7 Hz oscillations as the electrical manifestation of a non-convulsive status epilepticus (NCSE) would allow us to explain their widespread representation on the entire scalp either through their 'vertical' propagation via (cortico-) thalamo-cortical circuits/loops or their 'horizontal' propagation via intra- (e.g., superior longitudinal fasciculus) and inter-hemispheric (e.g., corpus callosum) cortico-cortical connections. The analysis of our data does not allow us to favor one of the two hypotheses, but rather it seems in agreement with them both. Indeed, the fact, that the 7 Hz peak shows a reduced amplitude on the right hemisphere, in correspondence of the lesional area (Fig. 4), could suggest a thalamo-cortical mechanism with a widespread projection mode, with a lower expression of the thalamic-driven oscillations on that area as a result of its neuronal damage; conversely, the graphical representation of the theta oscillations intrinsic connectivity within the examined networks (Fig. 5) would seem to carry some elements in favor of a propagation occurring especially through transcallosal, cortico-cortical connection systems.

Moreover, the EEG pattern of non-convulsive status epilepticus is not necessarily characterized by specific graphoelements (such as spike-and-wave patterns), but can be simply represented by slow oscillations (Smith, 2005). This hypothesis, finally, would seem

supported by the striking response to midazolam, a widely used drug for its anti-epileptic properties due to its powerful non-specific effects of neuronal firing inhibition and discharge spreading contrast (Hanley and Kross, 1998; Claassen et al., 2002; Gathwala et al., 2007).

On the other hand, there is another pathological form which shows a characteristic and critical response to benzodiazepine agonists GABA A including midazolam, that is catatonia. The facts that a) some cases of catatonia can be regarded as the manifestation of a NCSE, b) the EEG pattern shown by our patient was compatible with that of catatonia, and finally c) our patient's clinical picture was also compatible with the diagnosis of catatonia, are all elements potentially capable of binding together the NCSE and catatonia, at least for the case herein presented. Let us examine in detail the individual items.

The etiopathogenetic hypothesis which sees catatonia as the symptomatologic manifestation of a non-convulsive status epilepticus (NCSE) has long been known (Goldensohn and Gold, 1960; Lim et al., 1986; Louis and Pflaster,1995; Carroff et al., 2007; Daniels, 2009). This hypothesis is supported by the fact that catatonia characteristically and critically responds either to benzodiazepines or electroconvulsive therapy (ECT), both able also to raise the epileptic threshold. Our patient, as we have observed, showed a disappearance of 7 Hz oscillations after midazolam administration, in particular in those cortical regions (such as the orbitofrontal mesial cortex, the anterior cingulate cortex and the supplementary motor area) whose activation pattern is notoriously and characteristically altered in catatonic patients.

The EEG pattern of catatonic subjects is described in literature as widely slowed "most often in the theta frequency" (Smith et al., 2012) and the 7 Hz oscillations that, in our patient dominate in a widespread manner the EEG spectral profile, are fully included in the theta frequency band.

Finally, despite our patient's initial clinical diagnosis was that of MCS (+), a diagnostic hypothesis of catatonia has also been formulated with ex-adjuvantibus criterion, taking into account that a) until then no cases of midazolam-responsive MCS had been reported, whereas b) catatonia is known to respond to GABA A agonist drugs, including midazolam (McDonald et al., 2011). In fact, by administering ex post to our patient an appropriate diagnostic clinical scale, we have obtained a score compatible with a diagnosis of catatonia.

In this perspective, the recent description of a case of catatonia occurring after ablation of the right temporal lobe (Malur et al., 2010) acquires particular interest, reflecting the fact that dysfunction of this specific region of the brain may give rise to a picture of catatonia.

6. Concluding remarks

At this point, taking into account that functionally depressed regions in catatonia largely overlap those regions known as ipoactive in severe disorders of consciousness (DOC) (Laureys et al., 1999, 2006; Vanhaudenhuyse et al., 2010, Bruno et al., 2012), a twofold question arises: are we faced with a case of catatonia on an organic basis mimicking a case of MCS or does the MCS, as a syndromic entity in itself, also include elements of catatonic nature, whose relative weight in the individual patient will determine whether or not he/she will respond to GABA A agonist drugs? At present, we do not have enough information to answer these questions. However, at least for some patients with an initial diagnosis of MCS, we propose not to neglect such possibilities (Hem et al., 2005; Alisky, 2009).

Considering the MCS from this point of view could pave the way to new perspectives for both therapy and clinical management: at least a part of MCS patients could in fact benefit from treatment with non-selective GABA A agonists, especially if they show an EEG pattern characterized by a widespread 7 Hz peak. A first consequence of practical nature, is that such patients should be tested not only towards zolpidem but also towards benzodiazepines (i.e., not only with GABA A selective, but also with GABA A non-selective drugs), after the application of a sequential decision procedure including different test paradigms aiming at a reliable evaluation of cognitive functions in MCS patients as suggested by Kotchoubey and collegues (2013). In the case of a positive response to the test these patients may be candidates for a continued treatment with oral benzodiazepines or, theoretically, for a more aggressive protocol, such as the continuous intravenous infusion of midazolam, which is commonly practiced in the intensive care unit to interrupt a status epilepticus. In addition, in the future, given the growing evidence regarding the responsiveness of catatonia to rTMS (Kate et al., 2011), this latter method may prove to be potentially effective in 'awakening' also this type of patients.

Acknowledgments

The authors would like to thank Dr. Bruno Formichi and Dr. Giuseppe Arcidiacono for their anesthesiological supervision during midazolam administration. No funding has supported this study.

References

Alisky, J.M. (2009). Minimally-responsive head injury survivors may have chronic catatonia reversed years after an accident. *Singap Med J*, *50*(11), 1117-1118.

Andrews, R.J. (1991). Transhemispheric diaschisis. A review and comment. *Stroke*, *22*(7), 943-949.

Binder, J.R., Desai, R.H., Graves, W.W., & Conant, L.L. (2009). Where is the semantic system? A critical review and meta-analysis of 120 functional neuroimaging studies. *Cereb Cortex*, *19*(12), 2767-2796.

Bonfiglio, L., Olcese, U., Rossi, B., Frisoli, A., Arrighi, P., Greco, G., Carozzo, S., Andre, P., Bergamasco, M., & Carboncini, M.C. (2013). Cortical source of blink-related delta oscillations and their correlation with levels of consciousness. *Hum Brain Mapp*, *34*(9), 2178-2189.

Bonfiglio, L., Piarulli, A., Olcese, U., Andre, P., Arrighi, P., Frisoli, A., Rossi, B., Bergamasco, M., & Carboncini, M.C. (2014). Spectral parameters modulation and source localization of blink-related alpha and low-beta oscillations differentiate minimally conscious state from vegetative state/unresponsive wakefulness syndrome. *PLoS ONE*, *9*(3), e93252.

Brefel-Courbon, C., Payoux, P., Ory, F., Sommet, A., Slaoui, T., Raboyeau, G., Lemesle, B., Puel, M., Montastruc, J.L., Demonet, J.F., & Cardebat, D. (2007). Clinical and imaging evidence of zolpidem effect in hypoxic encephalopathy. *Ann Neurol*, *62*(1), 102-105.

Bruno, M.A., Majerus, S., Boly, M., Vanhaudenhuyse, A., Schnakers, C., Gosseries, O., Boveroux, P., Kirsch, M., Demertzi, A., Bernard, C., Hustinx, R., Moonen, G., & Laureys, S. (2012). Functional neuroanatomy underlying the clinical subcategorization of minimally conscious state patients. *J Neurol*, *259*(6), 1087-1098.

Canuet, L., Ishii, R., Pascual-Marqui, R.D., Iwase, M., Kurimoto, R., Aoki, Y., Ikeda, S., Takahashi, H., Nakahachi, T., & Takeda, M. (2011). Resting-State EEG Source Localization and Functional Connectivity in Schizophrenia-Like Psychosis of Epilepsy. *PloS ONE*, *6*(1), e27863.

Caradoc-Davies, T.H. (1996). Traumatic mutism in severe brain injury relieved by oral diazepam. *Disabil Rehabil*, *18*(9), 482-484.

Caroff, S.N., Mann, S.C., Francis, A., & Fricchione, G.L. (2004). Catatonia: From psychopathology to neurobiology. Arlington VA: American Psychiatric Publishing.

Claassen, J., Hirsch, L.J., Emerson, R.G., & Mayer, S.A. (2002). Treatment of refractory status epilepticus with pentobarbital, propofol, or midazolam: A systematic review. *Epilepsia*, *43*(2), 146-153.

Clauss, R.P., Güldenpfennig, W.M., Nel, H.W., Sathekge, M.M., & Venkannagari, R.R. (2000). Extraordinary arousal from semi-comatose state on zolpidem. A case report. *S Afr Med J*, *90*(1), 68-72.

Clauss, R.P., van der Merwe, C.E., & Nel, H.W. (2001). Arousal from a semi-comatose state on zolpidem. *S Afr Med J*, *91*(10), 788-789.

Clauss, R.P., & Nel, H.W. (2004). Effect of zolpidem on brain injury and diaschisis os detected by 99mTc HMPAO brain SPECT in humans. *Arzneimittelforschung*, *54*(10), 641-646.

Clemens, B., Bessenyei, M., Fekete, I., Puskás, S., Kondákor, I., Tóth, M., & Hollódy, K. (2010). Theta EEG source localization using LORETA in partial epilepsy patients with and without medication. *Clin Neurophysiol*, *121*(6), 848-858.

Daniels, J. (2009). Catatonia: Clinical aspects and neurobiological correlates. *J Neuropsychiatry Clin Neurosci*, *21*(4), 371-380.

Farge, M. (1992). Wavelet transforms and their applications to turbulence. *Annu Rev Fluid Mech*, *24*(1), 395-457.

Folstein, M.F., Folstein, S.E., & McHugh, P.R. (1975). Mini Mental State a practical method for grading the cognitive state of patients for the clinicians. *J Psychiat Res*, *12*(3), 189-198.

Fox, M.D., Snyder, A.Z., Vincent, J.L., Corbetta, M., Van Essen, D.C., & Raichle, M.E. (2005). The human brain is intrinsically organized into dynamic, anticorrelated functional networks. *P Natl Acad Sci USA*, *102*(27), 9673-9678.

Gathwala, G., Goel, M., Singh, J., & Mittal, K. (2012). Intravenous Diazepam, midazolam and Lorazepam in acute seizure control. *Indian J Pediatr*, *79*(3), 327-332.

Giacino, J.T., Ashwal, S., Childs, N., Cranford, R., Jennett, B., Katz, D.I., Kelly, J.P., Rosenberg, J.H., Whyte, J., Zafonte, R.D., & Zasler, N.D. (2002). The minimally conscious state Definition and diagnostic criteria. *Neurology*, *58*(3), 349-353.

Goldensohn, E.S., & Gold, A.P. (1960). Prolonged behavioural disturbances as ictal phenomena. *Neurology*, *10*, 1-9.

Grabenstatter, H.L., Russek, S.J., & Brooks-Kayal, A.R. (2012). Molecular pathways controlling inhibitory receptor expression. *Epilepsia*, *53*(9), 71-78.

Hanley, D.F., & Kross, J.F. (1998). Use of midazolam in the treatment of refractory status epilepticus. *Clin Ther*, *20*(6), 1093-1105.

Hem, E., Andreassen, O.A., Robasse, J.M., Vatnaland, T.R., & Opjordsmoen, S. (2005). Should catatonia be part of the differential diagnosis of coma? *Nord J Psychiatry*, *59*(6), 528-530.

Hindriks, R., & van Putten, M.J. (2013). Thalamo-cortical mechanisms underlying changes in amplitude and frequency of human alpha oscillations. *Neuroimage*, *70*, 150-163.

Jones Gotman, M. (1987). *Psychological evaluation-Testing hippocampal function*, In: Engel J, editor, Surgical Treatment of the Epilepsies. New York: Raven Press, pp. 203-215.

Juhasz, C., Kamondi, A., & Szirmai, I. (1997). Spectral EEG analysis following hemispheric stroke: Evidences of transhemispheric diaschisis. *Acta Neurol Scand*, *96*(6), 397-400.

Kang, H.J., Kawasawa, Y.I., Cheng, F., Zhu, Y., Xu, X., Li, M., Sousa, A.M., Pletikos, M., Meyer, K.A., Sedmak, G., Guennel, T., Shin, Y., Johnson, M.B., Krsnik, Z., Mayer, S., Fertuzinhos,

S., Umlauf, S., Lisgo, S.N., Vortmeyer, A., Weinberger, D.R., Mane, S., Hyde, T.M., Huttner, A., Reimers, M., Kleinman, J.E., & Sestan, N. (2011). Spatio-temporal transcriptome of the human brain. *Nature*, *478*(7370), 483-489.

Kate, M.P., Raju, D., Vishwanathanm, V., Khan, F.R. and Thomas, S.V. (2011). Successful treatment of refractory organic catatonic disorder with repetitive transcranial magnetic stimulation (rTMS) therapy. *J Neuropsychiatry Clin Neurosci*, *23*(3), E2-E3.

Kotchoubey, B., Veser, S., Real, R., Herbert, C., Lang, S., & Kübler, A. (2013). Towards a more precise neurophysiological assessment of cognitive functions in patients with disorders of consciousness. *Restor Neurol Neurosci*, *31*(4), 473-485.

Laureys, S., Lemaire, C., Maquet, P., Phillips, C., & Franck, G. (1999). Cerebral metabolism during vegetative state and after recovery to consciousness. *J Neurol Neurosurg Psychiatry*, *67*(1), 121-122.

Laureys, S., Boly, M., & Maquet, P. (2006). Tracking the recovery of consciousness from coma. *J Clin Invest*, *116*(7), 1823-1825.

Lehembre, R., Gosseries, O., Lugo, Z., Jedidi, Z., Chatelle, C., Sadzot, B., Laureys, S., & Noirhomme, Q. (2012). Electrophysiological investigations of brain function in coma, vegetative and minimally conscious patients. *Arch Ital Biol*, *150*(2-3), 122-139.

Lim, J., Yagnik, P., Schraeder, P., & Wheeler, S. (1986). Ictal catatonia as a manifestation of nonconvulsive status epilepticus. *J Neurol Neurosurg Psychiatry*, *49*(7), 833-836.

Louis, E.D., & Pflaster, N.L. (1995). Catatonia mimicking nonconvulsive status epilepticus. *Epilepsia*, *36*(9), 943-945.

Makeig, S., Bell, A.J., Jung, T.P., & Sejnowski, T.J. (1996). *Independent component analysis of electroencephalographic data*, In: Touretzky. D., Mozer, M., Hasselmo, M., editors. Adv Neur Inf, Vol 8, Cambridge, MA: The MIT Press, pp. 145-151.

Malur, C. and Clark, A. (2010).Catatonia after right temporal lobe resection. *J Neuropsychiatry Clin Neurosci*, *22*(3), 352.e17.

Matsuda, W., Komatsu, Y., Yanaka, K., & and Matsumura, A. (2005). Levodopa treatment for patients in persistent vegetative or minimally conscious states. *Neuropsych Rehabil*, *15*(3-4), 414-427.

Mazziotta, J., Toga, A., Evans, A., Fox, P., Lancaster, J., Zilles, K., Woods, R., Paus, T., Simpson, G., Pike, B., Holmes, C., Collins, L., Thompson, P., MacDonald, D., Iacoboni, M., Schormann, T., Amunts, K., Palomero-Gallagher, N., Geyer, S., Parsons, L., Narr, K., Kabani, N., Le Goualher, G., Boomsma, D., Cannon, T., Kawashima, R., & Mazoyer, B. (2001). A probabilistic atlas and reference system for the human brain: International Consortium for Brain Mapping (ICBM). *Philos Trans R Soc Lond B Biol Sci*, *356*(1412), 1293–1322.

McDonald, M.D., Larry, V., Liskow, M.D., & Barry, I. (2011). Reversal of Catatonia with Midazolam. *Jefferson Journal of Psychiatry*, *10*(2), 10.

Nichols, T.E., & Holmes, A.P. (2002). Nonparametric permutation tests for functional neuroimaging: A primer with examples. *Hum Brain Mapp*, *15*(1), 1-25.

Northoff, G., Kötter, R., Baumgart, F., Danos, P., Boeker, H., Kaulisch, T., Schlagenhauf, F., Walter, H., Heinzel, A., Witzel, T., & Bogerts, B. (2004). Orbitofrontal cortical dysfunction in akinetic catatonia: A functional magnetic resonance imaging study during negative emotional stimulation. *Schizophr Bull*, *30*(2), 405-427.

Nyakale, N.E., Clauss, R.P., Nel, W., & Sathekge, M. (2010). Clinical and brain Spect scan response to zolpidem in patients after brain damage. *Arzneimittelforschung*, *60*(4), 177-181.

Olbrich, S., Mulert, C., Karch, S., Trenner, M., Leicht, G., Pogarell, O., & Hegerl, U. (2009). EEG-vigilance and BOLD effect during simultaneous EEG/fMRI measurement. *Neuroimage*, *45*(2), 319-332.

Pascual-Marqui, R.D. (2002). Standardized low resolution brain electromagnetic tomography (sLORETA): Technical details. *Method Find Exp Clin Pharmacol*, *24*(Suppl D), 5-12.

Pascual-Marqui, R.D. (2007). Instantaneous and lagged measurements of linear and nonlinear dependence between groups of multivariate time series: Frequency decomposition. arXiv 0711.1455.

Passler, M.A., & Riggs, R.V. (2001). Positive outcomes in traumatic brain injury-vegetative state: Patients treated with bromocriptine. *Arch Phys Med Rehabil*, *82*(3), 311-315.

Pizzagalli, D., Pascual-Marqui, R.D., Nitschke, J.B., Oakes, T.R., Larson, C.L., Abercrombie, H.C., Schaefer, S.M., Koger, J.V., Benca, R.M., & Davidson, R.J. (2001). Anterior cingulate activity as a predictor of degree of treatment response in major depression: Evidence from brain electrical tomography analysis. *Am J Psychiat*, *158*(3), 405-415.

Qin, Y., Xu, P., & Yao, D. (2010). A comparative study of different references for EEG default mode network: The use of the infinity reference. *Clin Neurophysiol*, *121*(12), 1981-1991.

Sawyer, E., Mauro, L.S., & Ohlinger, M.J. (2008). Amantadine enhancement of arousal and cognition after traumatic brain injury. *Ann Pharmacother*, *42*(2), 247-252.

Scheuerecker, J., Ufer, S., Käpernick, M., Wiesmann, M., Brückmann, H., Kraft, E., Seifert, D., Koutsouleris, N., Möller, H.J., & Meisenzahl, E.M. (2009). Cerebral network deficits in post-acute catatonic schizophrenic patients measured by fMRI. *J Psychiatr Res*, *43*(6), 607-614.

Schiff, N.D. (2008). Central thalamic contributions to arousal regulation and neurological disorders of consciousness. *Ann NY Acad Sci*, *1129*(1), 105-118.

Schiff, N.D. (2010). Recovery of consciousness after severe brain injury: The role of arousal regulation mechanisms and some speculation on the heart-brain interface. *Clev Clin J Med*, *77*(Suppl 3), S27-S33.

Shames, J.L., & Ring, H. (2008). Transient reversal of anoxic brain injury-related minimally conscious state after zolpidem administration: A case report. *Arch Phys Med Rehabil*, *89*(2), 386-388.

Šidák, Z.K. (1967). Rectangular confidence regions for the means of multivariate normal distributions. *J Am Stat Assoc*, *62*(318), 626-633.

Smith, S.J.M. (2005). EEG in the diagnosis, classification, and management of patients with epilepsy. *J Neurol Neurosurg Psychiatry*, *76*(Suppl 2), ii2-ii7.

Smith, J.H., Smith, V.D., Philbrick, K.L., & Kumar, N. (2012). Catatonic disorder due to a general medical or psychiatric condition. *J Neuropsychiatry Clin Neurosci*, *24*(2), 198-207.

Tagliaferri, F., Compagnone, C., Korsic, M., Servadei, F., & Kraus, J. (2006). A systematic review of brain injury epidemiology in Europe. *Acta Neurochir*, *148*(3), 255-268.

Taira, T., & Hori, T. (2007). Intrathecal baclofen in the treatment of post-stroke central pain. dystonia and persistent vegetative state. *Acta Neuroch Supp*, *97*(Pt 1), 227-229.

Thurman, D., & Guerrero, J. (1999). Trends in hospitalization associated with traumatic brain injury. *JAMA*, *282*(10), 954-957.

Torrence, C., & Compo, G.P. (1998). A practical guide to wavelet analysis. *Bull Am Math Soc*, *79*(1), 61-78.

Towle, V.L., Bolanos, J., Suarez, D., Tan, K., Greszczuk, R., Levin, D.N., Cakmur, R., Frank, S.A., & Spire, J.P. (1993). The spatial location of EEG electrodes: Locating the best fitting sphere relative to cortical anatomy. *Electroencephalogr Clin Neurophysiol*, *86*(1), 1-6.

Valdés-Hernández, P.A., von Ellenrieder, N., Ojeda-Gonzalez, A., Kochen, S., Alemán-Gómez, Y., Muravchik, C., & Valdés-Sosa, P.A. (2009). Approximate average head models for EEG source imaging. *J Neurosci Meth*, *185*(1), 125-132.

Vanhaudenhuyse, A., Noirhomme, Q., Tshibanda, L.J.F., Bruno, M.A., Boveroux, P., Schnakers, C., Soddu, A., Perlbarg, V., Ledoux, D., Brichant, J.F., Moonen, G., Maquet, P., Greicius, M.D., Laureys, S., & Boly, M. (2010). Default network connectivity reflects the level of consciousness in non-communicative brain-damaged patients. *Brain*, *133*(Pt 1), 161-171.

Victor, J.D., Drover, J.D., Conte, M.M., & Schiff, N.D. (2011). Mean- field modeling of thalamocortical dynamics and a model-driven approach to EEG analysis. *Proc Natl Acad Sci USA*, *108*(Suppl 3), 15631-15638.

Whyte, J., Katz, D., Long, D., DiPasquale, M.C., Polansky, M., Kalmar, K., Giacino, J., Childs, N., Mercer, W., Novak, P., Maurer, P., & Eifert, B. (2005). Predictors of outcome in prolonged posttraumatic disorders of consciousness and assessment of medication effects: A multicenter study. *Arch Phys Med Rehabil*, *86*(3), 453-462.

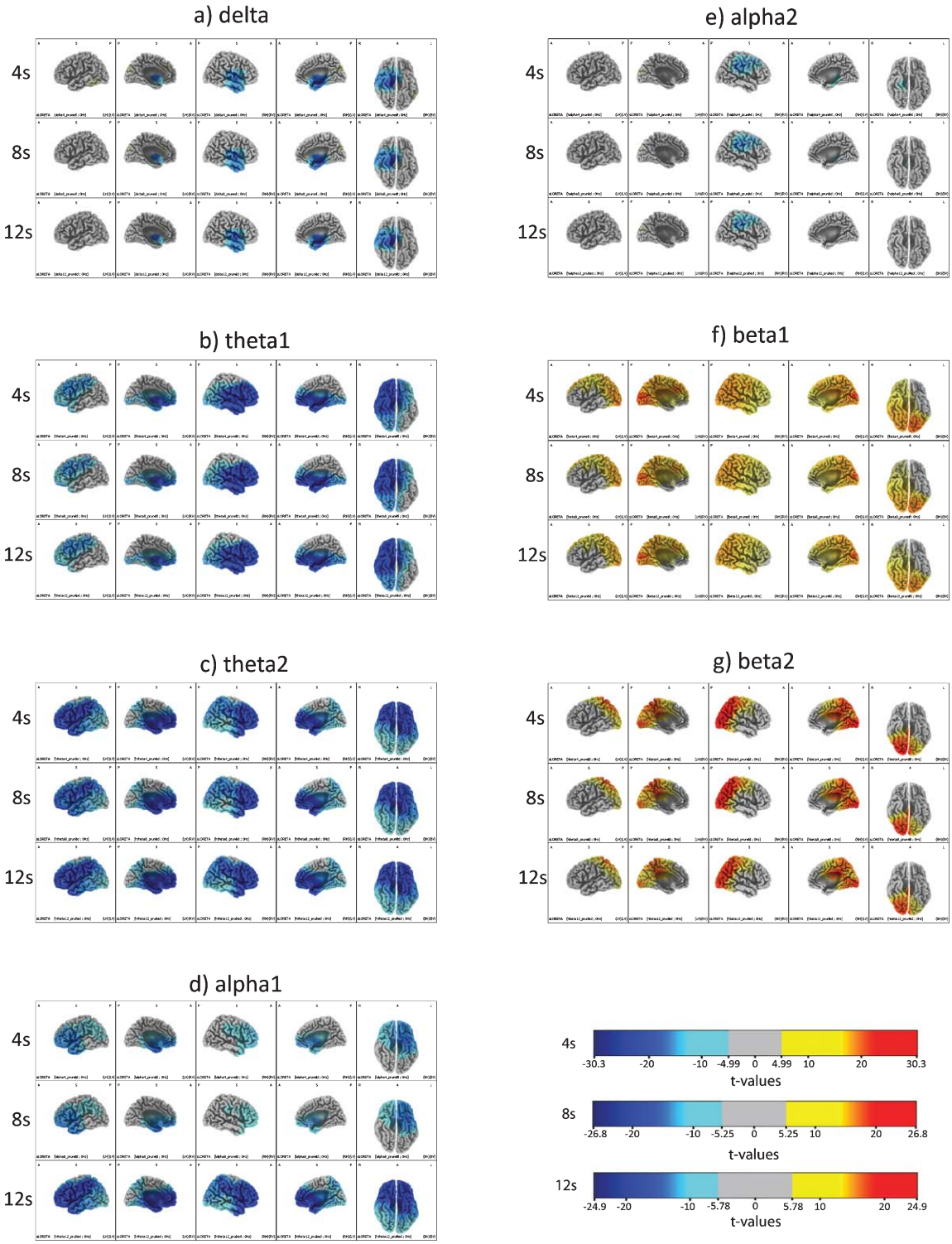
Whyte, J., & Myers, R. (2009). Incidence of clinically significant responses to zolpidem among patients with disorders of consciousness: A preliminary placebo controlled trial. *Am J Phys Med Rehabil*, *88*(5), 410-418.

Williams, S.T., Conte, M.M., Goldfine, A.M., Noirhomme, Q., Gosseries, O., Thonnard, M., Beattie, B., Hersh, J., Katz, D.I., Victor, J.D., Laureys, S., & Schiff, N.D. (2013). Common resting brain dynamics indicate a possible mechanism underlying zolpidem response in severe brain injury. *eLife*, *2*, e01157.

Yao, D., Wang, L., Oostenveld, R., Nielsen, K.D., Arendt-Nielsen, L., & Chen, A.C. (2005). A comparative study of different references for EEG spectral mapping: The issue of the neutral reference and the use of the infinity reference. *Physiol Meas*, *26*(3), 173-184.

Yao, D., Wang, L., Arendt-Nielsen, L., & Chen, A.C. (2007). The effect of reference choices on the spatio-temporal analysis of brain evoked potentials: The use of infinite reference. *Comput Biol Med*, *37*(11), 1529-1538.

Supplementary Material



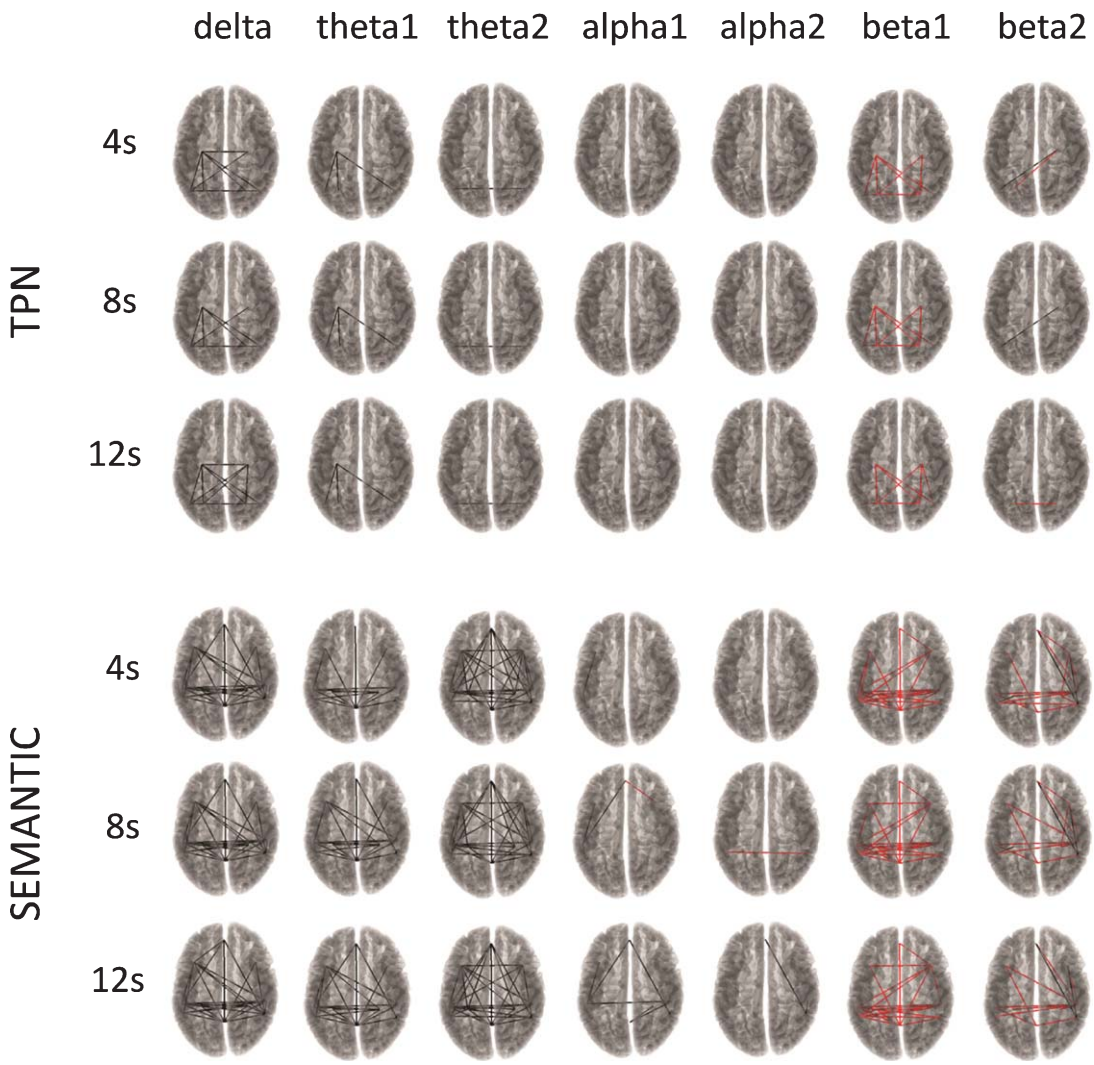


Fig. B. Significant differences of phase synchronization are presented for two networks of interest (Task Positive Network, TPN, and Semantic Network, SN). Black tones indicate significantly higher phase synchronization during the pre-infusion (overall $p < 0.01$) whereas red tones indicate significantly higher levels during the post-infusion (overall $p < 0.01$). Rows refer respectively to 4, 8 and 12 seconds epoching. Also in this case, significant results are consistent across different epoching choices.

Fig. A. Voxels with significantly different levels of activation between post and pre-infusion, are presented for each of the 7 bands. Panels refer to delta, theta1, theta, alpha1, alpha2, beta1 and beta2 respectively. Yellow to red tones correspond to progressively higher (i.e. with higher *t*-values) activations in the post-infusion whereas light to dark blue tones indicate a progressively higher activation in the pre-infusion. First column of each panel represents a left view of the left hemisphere, second column a right view of the left hemisphere, third column a right view of the right hemisphere, fourth column a left view of the right hemisphere and fifth column a view from below of the whole cortex. In each panel the first row refers to analyses performed on 4 seconds epochs, the second row to 8 seconds epochs and the third to 12 seconds epochs. Last panel shows the colorbars referred to the three different epoching. In each colorbar the grey-colored area indicates *t*-values corresponding to *p*-values higher than 0.01. Note that results are substantially independent from the epoching choice.