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Probing ERP correlates of verbal semantic processing in patients with impaired consciousness

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ABSTRACT

Our ability to identify covert cognitive abilities in non-communicating patients is of prime importance to improve diagnosis, to guide therapeutic decisions and to better predict their cognitive outcome. In the present study, we used a basic and rigorous paradigm contrasting pairs of words orthogonally. This paradigm enables the probing of semantic processing by comparing neural activity elicited by similar words delivered in various combinations. We describe the respective timing, topography and estimated cortical sources of two successive event-related potentials (ERP) components (N400 and late positive component (LPC)) using high-density EEG in conscious controls ($N=20$) and in minimally conscious (MCS; $N=15$) and vegetative states (VS; $N=15$) patients recorded at bedside. Whereas N400-like ERP components could be observed in the VS, MCS and conscious groups, only MCS and conscious groups showed a LPC response, suggesting that this late effect could be a potential specific marker of conscious semantic processing. This result is coherent with recent findings disentangling early and local non-conscious responses (e.g.: MMN in odd-ball paradigms, N400 in semantic violation paradigms) from late, distributed and conscious responses (e.g.: P3b to auditory rule violation) in controls and in patients with disorders of consciousness. However, N400 and LPC responses were not easily observed at the individual level, – even in conscious controls – , with standard ERP analyses, which is a limiting factor for its clinical use. Of potential interest, the only 3 patients presenting both significant N400 and LPC effects were MCS, and 2 of them regained consciousness and functional language abilities.

1. Introduction

The objective evaluation of cognitive abilities of non-communicating patients is one of the most challenging current medical issues. Such an evaluation is of prime importance to guide acute therapeutic decisions, to improve prognosis determination, and to inform patients' relatives. An expert, detailed and repeated clinical examination of patients, associated with the use of dedicated behavioral scales, is the best current approach. Over the last decades, new behavioral scales have been developed, aiming at detecting the emergence from comatose state (Giacino et al., 2004; Wijdicks et al., 2005), and at differentiating patients in the vegetative state from those in conscious or minimally conscious states (Fins et al., 2007). A recent assessment of these clinical and behavioral methods demonstrated that these scales importantly reduced diagnosis errors by 30–45% (Andrews et al., 1996; Schnakers et al., 2009).

While this approach remains necessary, it can only assess *overt* behaviors. In the absence of behavioral response, a second and complementary approach should thus aim at detecting *covert* cognitive abilities directly from patient's brain activity. For instance, Owen and colleagues elaborated a mental imagery task during which the patient is instructed to imagine playing tennis or walking in his home (Owen et al., 2006). This task, which requires the combination of verbal, working memory and mental imagery skills, is thought to require conscious processing (Naccache, 2006). A few clinically VS patients showed this same pattern of activation, which may be considered as an evidence for covert conscious processing (Monti et al., 2010). Following a parallel approach, we

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developed an active paradigm in which patients are asked to detect violations of auditory regularities. Crucially, our test enables us to disentangle early automatic brain responses to violations of short-range within-trials regularities (e.g. mismatch negativity, or MMN) which occur even in unconscious patients (Fischer et al., , 2004; Kane et al., 1996; Naccache et al., 2005b), from late strategic responses to violations of long-range between-trial rules (P3b response) (Bekinschtein et al., 2009). In conscious controls, the latter response requires conscious access to the between-trial rule. In patients, the occurrence of this late response was remarkably specific to clinically conscious or minimally conscious patients, as opposed to patients in VS (Faugeras et al., 2012). Indeed, similarly to the studies of Owen and colleagues, we identified two clinically VS patients who showed this response to violations of the long-range auditory rule (Faugeras et al., 2011). These two patients improved to a MCS a few days after the recordings, suggesting that we had captured signs of covert conscious processing in advance of clinical observation. We used multivariate pattern analysis techniques to improve the sensitivity of our test (King et al., 2013). However, all those tests still lack sensitivity, as evidenced by the many patients who are clinically conscious but nevertheless fail to show fMRI or EEG indices of conscious processing. The reasons for such poor sensitivity include fluctuations of vigilance, which are usual in such patients, as well as associated cognitive impairments. For instance, language impairments would impede comprehension of task instructions, whereas working memory deficits would prevent the active maintenance of the task set.

Therefore, an ideal goal would be to probe each of the patients' major cognitive modules, yielding a full neuropsychological profile, as is usual in communicating patients.

1.1. Scalp ERP signatures of verbal semantic processing: a two-stage model hypothesis

In the present work, we were interested in elaborating an ERP test probing verbal semantic processing. In 1980, Kutas and colleagues first discovered the N400, a scalp ERP event indexing violations of semantic congruity in visual or auditory sentences (Kutas and Hillyard, 1980). Since then, a rich literature investigated the precise psychological and neural properties of the N400 and of other correlates of semantic processing such as the early left anterior negativity (ELAN), or the late positive complex (LPC, also described as P600) (Kutas and Federmeier, 2011; Pulvermüler et al., 2009). The detailed description of this literature is clearly out of the scope of this experimental article, but it is noteworthy to mention the absence of consensual theoretical interpretation of the functional significance of each of these markers. While some theorists proposed to link the N400 with a late post-recognition stage of word processing (Brown and Hagoort, 1993), other models postulated that it reflects an early stage occurring prior to word recognition and semantic access (Deacon et al., 2004). In the current study we aimed at testing another model inspired by our previous works on conscious access and unconscious processing (Bekinschtein et al., 2009; Dehaene et al., 2006; Dehaene and Naccache, 2001; Gaillard et al., 2009). We previously showed, both in the visual and auditory modalities, that stimulus perception could be described as a two-stage model. While the first stage occurs in the absence of conscious access, and mostly engage stimulus processing in specialized perceptual networks, the second and later stage of processing is specifically associated with conscious access. This late stage would correspond to the broadcasting of the initial representation to a brain-scale distributed "global workspace" network (Baars, 1993; Bekinschtein et al., 2009; Dehaene et al., 2006). We and others, previously showed how this model can capture many empirical behavioral and functional neuro-imaging findings using various paradigms (e.g.: visual masking; attentional blink; neglect; distraction) and various brain-imaging tools (fMRI, high-density scal ERPs, stereo-electro-encephalography (SEEG), magneto-encephalography (MEG)) both in normal subjects and in neurological (e.g.: vegetative stage or minimally conscious patients (Bekinschtein et al., 2009; Faugeras et al., 2011, 2012; King et al., 2013); neglect patients (Sackur et al., 2008) and schizophrenic patients patients (Dehaene et al., 2003; Del Cul et al., 2006). Irrespectively of the specific stimulus attribute under consideration, the first stage would correspond to an early negativity (e.g.: N100, N200, MMN, N400), while the second and later stage of processing would be linked to a late P3b positive complex (Sergent et al., 2005; Van Gaal et al., 2014). Applied to the issue of word semantics, our theoretical approach proposes that semantic processing of words should follow the same two-stage model. The N400 would index the first non-conscious stage of semantic processing, whereas a late P3b-like complex would be the neural signature of conscious semantic processing. Indeed, several studies demonstrated that semantic processing of visual words can occur unconsciously in conscious subjects. For instance, when using a rapid-serial visual presentation (RSVP) task such as the 'attentional blink' paradigm, subjects failed to report target words, while a N400 signature of verbal semantic processing could still be observed (Luck et al., 1996). Sergent and Dehaene replicated this finding and further showed that while the N400 could occur in the absence of conscious access to the target word, a later event (P3b) was observed exclusively when subjects were conscious of this word (Sergent et al., 2005). Kiefer (2002) found a similar result using a masked semantic priming paradigm. These studies converge with those obtained in the auditory modality (see above) by finding a P3b component associated with conscious access. In one masking study, Naccache et al. even revealed a modulation of amygdala activity by the emotional valence of masked words in epileptic patients implanted with intracranial electrodes (Naccache et al., 2005a). Interestingly, while masked words elicited a single response in the amygdala, consciously perceived unmasked words elicited two successive responses, in agreement with our 2-stage model. In a recent ERP study investigating the semantic integration of multiple words in a visual masking paradigm, we showed that the N400 effects were similar for both masked and unmasked conditions, whereas the LPC/P600 effects were strongly affected by stimulus visibility (Van Gaal et al., 2014). Such qualitative differences are supporting our hypothesis that while the N400 is a marker of non-conscious semantic processing, the LPC/P600 indexes conscious semantic processing of words. Interestingly, other studies reported the presence of an N400 and the absence of P3b for unconsciously perceived words in the attentional blink paradigm (Luck et al., 1996; Sergent et al., 2005). Applying this 2-stage model of perception to word semantic attributes, we predicted that N400 could be observed both in conscious subjects (controls), and in DOC patients (MCS and VS), but that the LPC/P600 would be present exclusively in conscious subjects (conscious controls and some minimally conscious patients), but not in VS patients.

1.2. Verbal semantic processing in non-communicating patients

Several approaches have been developed to explore linguistic abilities in non-communicating patients. First, several studies explored brain responses to the patient's own name, a unique and extremely self-relevant word. Perrin et al. detected a P300 response to this self-related stimulus during sleep (Bastuji et al., 2002), but most crucially also in 6 out of 6 MCS patients and in 3 out of 5 VS patients (Perrin et al., 2006). Fischer et al. even reported this response in 21 out of 50 comatose patients (Fischer et al., 2008). Note however that cognitive processing of this single

stimulus is not easily interpretable: it may well reflect semantic content, but also familiarity and emotional processes. A second approach consists in using fMRI contrasts between verbal and non-verbal auditory stimuli. Schiff and colleagues revealed activations of widely distributed cortical systems in some MCS patients in response to meaningful language compared to meaningless time-reversed stimuli (Schiff et al., 2005). Davis et al. designed a hierarchy of contrasts probing different stages of semantic processing, including the perception of ambiguous words within a contextual sentence (Davis et al., 2007). During propofol sedation, superior temporal areas were still responding to sentences versus noise. However, the additional inferior frontal and posterior temporal activations observed in conscious subjects in response to ambiguous versus non-ambiguous sentences were absent. Using a similar paradigm, the same group scanned non-communicating patients (Coleman et al., 2007; Owen et al., 2005). Two conscious patients showed preserved speech processing of both low-ambiguity and high-ambiguity stimuli. Crucially, 3 out of 7 VS patients demonstrated some evidence of preserved speech processing.

We will now focus exclusively on patients' studies conducted with ERP recordings. To date, six studies used sentences or word-pairs paradigms to record ERP correlates of semantic processing in patients suffering from disorders of consciousness. Schoenle et al. examined 120 patients with severe brain damage, classified into three diagnostic groups: patients in VS, patients in 'near vegetative state', and patients not in vegetative state (Schoenle and Witzke, 2004). While VS patients as a group were least likely to show N400, approximately 12% of VS patients showed a semantic N400 response. Kotchoubey and colleagues explored 50 patients in permanent VS and could identify a significant N400 response in about 20% of the population (Kotchoubey et al., 2005). Two studies reported N400 responses in comatose patients (Kotchoubey et al., 2005; Rämä et al., 2010). In particular, Rämä and colleagues showed that while a group of comatose patients ($n=7$) exempt from temporal lobe lesions showed a N400 response at the group-level, no N400 could be observed in a group of comatose patients suffering from temporal lobe lesions. Note that 4/6 patients with temporal lesions had a right temporal lesion. (Steppacher et al., 2013) recently reported an ERP study of semantic congruity conducted in 92 patients (53 VS and 39 MCS patients) with an additional measure of clinical outcome between 2 and 14 years after discharge from rehabilitation. They found signs of semantic processing in 32% of VS patients, and most importantly they reported a clear association between such ERP response and prognosis outcome, both in MCS and in VS patients. Note however that the use of only 5 electrodes did not allow for a distinction between the several ERP components previously described in the literature, such as N400 and LPC. Finally, Balconi et al. recorded ERPs during a semantic associative task in eighteen patients classified as VS or MCS, and in 20 controls (Balconi et al., 2013). A N400 effect was observed in the patients group, with a delayed latency in patients as compared to the controls group. Moreover, no clear difference was found at the group level between VS and MCS patients.

In the present study we assessed the presence of the two main ERP correlates of verbal semantic processing (N400 and LPC) in controls and in non-communicating patients suffering from disorders of consciousness (DOC). We tested VS and MCS patients and evaluated their outcome in terms of functional communication recovery. We also aimed at comparing results of this auditory verbal semantic task with a variant of the auditory odd-ball paradigm (the 'local–global' task) which we use routinely as a test of conscious processing (Bekinschtein et al., 2009; Faugeras et al., 2011, 2012; King et al., 2013). Finally, we designed our study so to perform not only group-level, but also individual-level analyses, in order to assess clinical value of our technique.

2. Methods

2.1. Controls

Twenty right-handed native French speakers volunteered to this study. One of them could not be recorded due to excessively high impedances. The remaining subjects (mean age=29 years ±7.3; sex ratio=6 males/13 females) had no neurological or psychiatric history, were free of any medication and had normal or corrected to normal vision. All participants gave written informed consent, and the experiment was approved by the Ethical Committee of the Kremlin-Bicêtre hospital (n. 98-25).

2.2. Patients

Patients were recorded (between 2008 and 2012) in distinct Intensive Care Units (ICUs) of the Pitié-Salpêtrière hospital (Paris, France). Only patients who underwent a first ERP evaluation probing automatic and conscious processing of the auditory environment (Bekinschtein et al., 2009; Faugeras et al., 2011, 2012; Naccache et al., 2005b) were included in the present study. Following this recording, which was prescribed by the clinicians in charge of the patients, we recorded them with the auditory verbal semantic paradigm (recording time was increased by 20 min). This experiment was approved by the local Ethical committee (Pitié-Salpêtrière hospital). At recording time, patients were free of any sedation. ERP acquisition was systematically preceded by a detailed clinical evaluation adapted to DOC patients: standard neurological examination, Glasgow coma scale (GCS), FOUR score (Wijdicks et al., 2005) and Coma Recovery Scale-Revised scorings (CRS-R) (Giacino et al., 2004). Patients' outcome was assessed using the Glasgow Outcome Scale Extended (GOS-E) (Jennett et al., 1981), and the CRS-R communication sub-score (0=no communication, 1 intentional but no functional communication, 2 functional communication), with a 12-months follow-up. Positive outcomes were defined by recovery of a functional communication (which also implied being conscious, and not VS or MCS).

2.3. Auditory stimulation paradigms

We designed a simple semantic priming paradigm appropriate for patients with potentially severe cognitive impairments. Each trial consisted in the presentation of a pair of semantically related or unrelated auditory words. The first (prime) word acted as a semantic inductor for the second (target) word. From a French corpus of free word association (Alario and Ferrand, 1998; Ferrand, 2001), we extracted 68 pairs of associated words. Mean free word association rate for congruent pairs was 50% (see Table 1 and Tables S1 and S2 and auditory stimuli in SOM for details). In order to exclude any confound of the congruent/incongruent factor of interest with specific words, those 68 congruent pairs were spliced to construct 68 incongruent word-pairs. For instance, the congruent pairs "*sled-snow*" and "*hive-bee*" were spliced to build two incongruent pairs "*sled-bee*" and "*hive-snow*". Crucially, given that the very same elementary words were used across congruent and incongruent, the comparison of congruent and incongruent ERPs is exclusively dependent on the semantic priming effect (see Fig. 1 and Supplementary material). Stimuli were presented through earphones (Sennheiser HD 429), using Eprime v1.1 (Psychology Software Tools Inc., Pittsburgh, PA), in a quiet room for healthy volunteers, and in the intensive care bed for patients. All subjects, – including patients – , were instructed to passively listen to pairs of spoken words and to take some precautions to limit ocular artefacts ("keep your eyes closed and try to avoid any movement"). For patients who often kept their eyes open, an eye-cover was used in order to tend to equalize photic stimulation across blocks and across patients.

In every trial, first word (prime) duration was set to 467 ms, and was then followed 400 ms later by the second word (duration ranging from 173 ms to 585 ms), corresponding to a word1–word2 stimulus onset asynchrony of 867 ms. We equated stimulus duration to 467 ms for each first word in order to keep a constant intra-trial SOA between first and second words onsets across all pairs and trials. Note that all first words had a final uniqueness point. Therefore, both acoustic and semantic onsets were time-locked across trials. The words were all intelligible (stimuli are available as SOM). Inter-trial interval randomly varied from 2300 to 2800 ms with steps of 100 ms. Each block contained the randomly delivered 136 words pairs. Controls and patients have been exposed to 2–4 blocks, depending on recording impedance conditions (cut-off < 100 kΩ) and online artefacts, in order to tend to obtain a reasonable number of EEG valid trials. As previously mentioned, all patients were also recorded under the active counting version of the 'local–global' paradigm which captures early cortical auditory responses (P1), as well as MMN and late P3 complex (Bekinschtein et al., 2009). During this first ERP session, stimuli consisted of four similar sounds (1000 or 2000 Hz with duration of 50 ms) followed by either an identical (local standard trial) or a different fifth sound (local deviant trial). Inter-stimuli interval was 100 ms (for a detailed description see (Bekinschtein et al., 2009). In conscious controls, local regularity violation (local-deviant minus local standard trials) elicits a mismatch negativity (MMN) response often followed by a transient P3a

Table 1
Lexical characteristics of primes and targets.

Lexical characteristics	Primes ($n=68$)	Targets ($n=68$)
Grammatical category (verb/name/adverb/adjective)	0/57/0/11	2/53/1/12
Concreteness (%)	47.05	38.23
Gender (masculine/feminine/neuter)	14/49/5	35/26/7
Number (singular/plural)	67/1	60/0
Movie frequency (per million of occurrence; mean (SD))	55.85 (120.18)	141.87 (168.33)
Book frequency (per million of occurrence; mean (SD))	66.60 (140.66)	141.20 (168.32)
Homograph number (mean (SD))	1.34 (0.61)	1.62 (0.71)
Homophone number (mean (SD))	3.31 (1.96)	5.16 (3.78)
Letter number (mean (SD))	6.22 (1.57)	5.00 (1.21)
Syllable number (mean (SD))	1.72 (0.61)	1.46 (0.58)
Phoneme number (mean (SD))	4.34 (1.27)	3.74 (1.17)
Orthographic uniqueness point (mean (SD))	5.71 (1.60)	4.90 (1.19)
Phonological uniqueness point (mean (SD))	4.19 (1.19)	3.74 (1.17)
Orthographic neighbors (mean (SD))	2.78 (3.51)	5.57 (4.95)
Phonological neighbors (mean (SD))	7.88 (7.62)	12.50 (8.34)
Orthographic neighborhood[a] (mean (SD))	1.90 (0.44)	1.58 (0.32)
Phonological neighborhood[a] (mean (SD))	1.51 (0.43)	1.30 (0.33)

Adapted from Matos et al. (2001).

[a] Levenshtein's distance; SD=standard deviation.

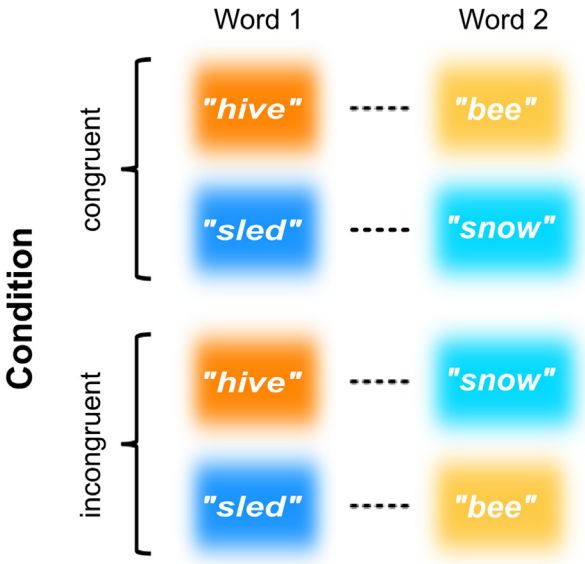

Fig. 1. Experimental design & usual N400 effect. On each trial, 2 words were sequentially presented with a fixed stimulus onset asynchrony of 867 ms. Word-pairs were either semantically congruent (e.g. *"hive-bee"*) or incongruent (e.g. *"hive-snow"*).

component (both responses are regrouped under the generic term of "local effect"). In addition to this intra-trial regularity effect, an inter-trial auditory rule was defined at the beginning of each block by the repetition of the same trials (e.g. local deviant or local standard). Patients were verbally instructed to pay attention to this structure, and to mentally count all violations of it. In conscious subjects and conscious patients, the detection of this rule violation (global-deviant trials minus global-standard trials) elicits a P3b-like potential which we called the 'global effect', which provides a very specific signature of conscious processing (Bekinschtein et al., 2009; Faugeras et al., 2011, 2012).

2.4. EEG recording and processing

EEG was sampled at 250 Hz with a 256-electrode geodesic sensor net connected to a high impendence amplifier (EGI, Oregon, USA) referenced to the vertex. Impedances were controlled inferior to 100 kΩ. Data were filtered from 0.5 Hz to 20 Hz. For the semantic paradigm, trials were segmented from −200 ms to 1000 ms relative to the onset of the second word. For the 'local–global' paradigm, trials were segmented from −200 ms to +1300 ms relative to the onset of the first of the five sounds.

Trials with voltage exceeding ± 100 μV or electro-oculogram activity exceeding ± 70 μV, or containing eye-blinks were rejected. Trials with more than 10/256 bad channels were rejected. For the remaining trials, bad channels were interpolated from contiguous electrodes. Remaining trials were averaged in synchrony with respective stimulus onset, digitally transformed to an average reference, and corrected for baseline over a 200 ms window before stimulus onset for the semantic paradigm, and over an 800 ms window for the 'local–global' paradigm. All pre-processing stages were performed in the EGI Waveform Tools Pack. Voltage topographical maps were plotted with Cartool software programmed by Denis Brunet (http://brainmapping.unige.ch/Cartool.htm).

Cortical current source density mapping was obtained using a distributed model consisting of 10,000 current dipoles. Dipole locations and orientations were constrained to the cortical mantle of a generic brain model built from the standard brain of the Montreal Neurological Institute using the BrainSuite software package. This head model was then warped to the standard geometry of the sensor net. The warping procedure and all subsequent source analysis were processed with the BrainStorm software package (http://neurimage.usc.edu/brainstorm). EEG forward modeling was computed with an extension to EEG of the overlapping-spheres analytical model. Cortical current maps were computed from the EEG time series using a linear inverse estimator (weighted minimum-norm current estimate or WMNE, see (Tadel et al., 2011) for review). We computed sources of the grand-average calculated in controls (incongruent pairs minus congruent pairs). Source estimations were converted in *Z*-score in comparison with a 200 ms long baseline window preceding the onset of the second word.

2.5. Statistical analyses

We used the same method as previously reported in our previous publications on the 'local–global' paradigm (Bekinschtein et al., 2009; Faugeras et al., 2011, 2012).

Group analyses were computed using sample-by-sample paired *t*-tests with a triple criterion: *t*-test *p* value was categorized in three levels (non-significant, $0.01 \leq p < 0.05$ or $p < 0.01$), for a minimal duration of 10 consecutive samples (40 ms) at least on 10 electrodes.

A region of interest (ROI) approach was also used by computing sample-by-sample paired t-tests on the mean signal averaged across 10 contiguous electrodes centered on the spatial maximum of the effect in the controls group. Peaks of the ERP effects were calculated as the maximal difference between incongruent and congruent averaged ROIs ERPs. Note that this method is circular when evaluating statistical significance of an ERP effect when applied to the controls group due to a "double-dipping" issue (Kriegeskorte et al., 2009), but is valid when exploring patients' groups, and individual-subject data.

In order to take advantage of the high-spatial resolution we supplemented the electrode-by-electrode and ROI analyses with a multiple-linear spatial regression approach able to exploit scalp topographies of voltages (Pegado et al., 2010). N400 and LPC effect were defined by a 257-values vector corresponding to the averaging of voltages during the relevant time-window in controls subjects (436–516 ms for N400; 652–1000 ms for LPC, see Fig. 2b). Then for each patient group (DOC, VS and MCS subgroups) voltage time series were regressed with a model including the effects of interest and a constant regressor. For each group of patients, distributions

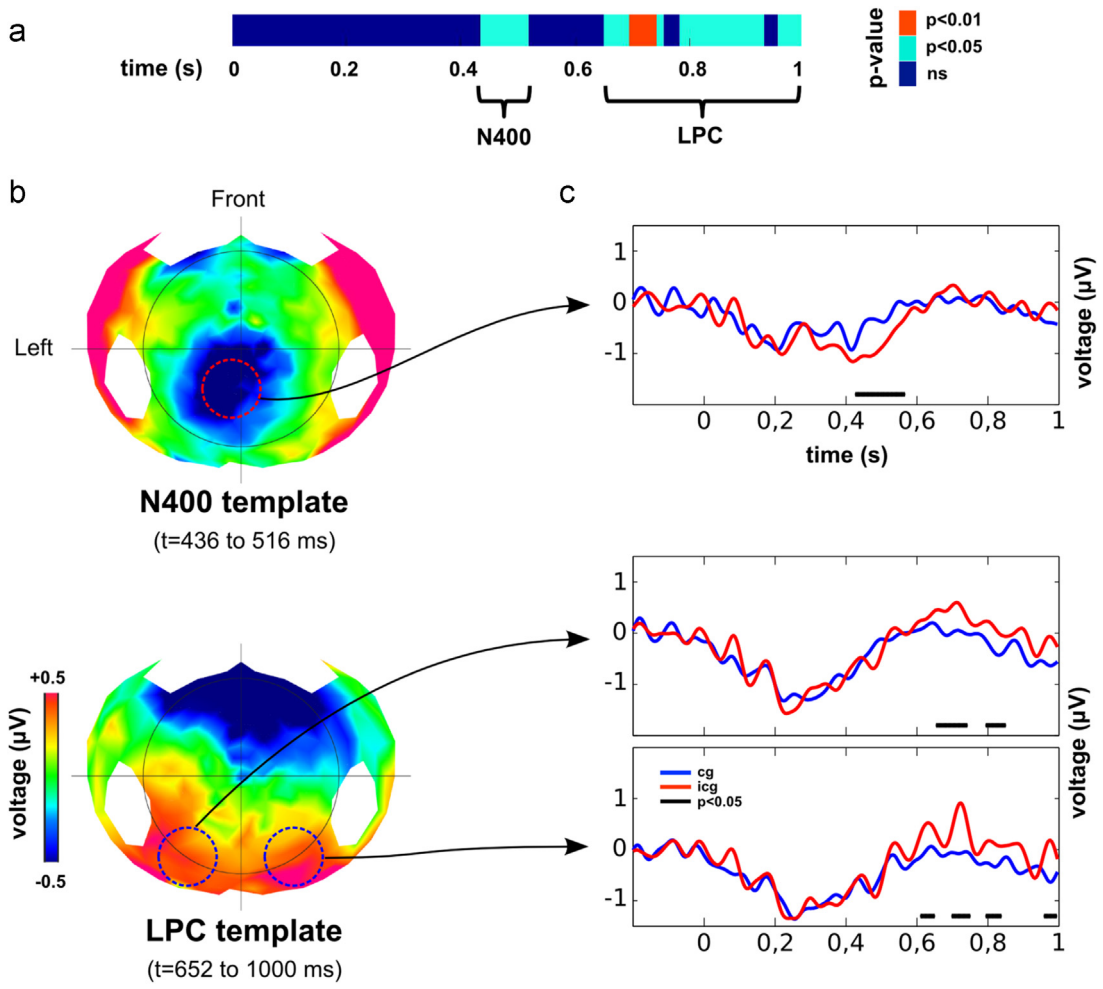

Fig. 2. N400 and LPC in controls (a) overview of semantic congruity effects in the controls group using the triple threshold paired *t*-tests approach (see section Methods). Two significant periods were found, corresponding respectively to the N400 and to the LPC. (b) N400 and LPC templates defined in this controls group and used for linear regression on patients' group and individual data. These voltage topographies were calculated by the subtraction (incongruent–congruent) in the N400 and LPC time windows. (c) ERPs for incongruent and congruent conditions computed in 3 regions of interest (note that this result is circular, and is only shown for a descriptive purpose in the perspective of patients' data analysis).

of coefficients of interest were tested against the null hypothesis with an unpaired *t*-test ($p < 0.05$ which correspond to a *t*-value > 1.96).

Individuals' statistics were computed using a sample-by-sample unpaired *t*-test between experimental conditions across trials with a triple criterion: $p \leq 0.05$ on a minimum of 5 consecutive samples (20 ms) and on a minimum of 10 electrodes. In order to further assess the power of observed effects, we categorized the significance of the semantic effect for each time-sample using a 6-level *p*-value scale: ≤ 0.05, ≤ 0.01, ≤ 0.005, ≤ 0.001, ≤ 0.0005 and ≤ 0.0001. A last correction was then used on each recording in order to increase the specificity of our analyses. On the basis of the group analysis, we categorized a semantic congruity effect as a N400 if its onset ranged from 200 to 600 ms after the onset of the second word, and as a LPC if its latency ranged from 600 to 1000 ms. All *p*-values of interest (200–1000 ms after the onset of the second word) superior to the lowest *p*-values observed in this recording within the baseline time-window and the first 200 ms (-200 to $+200$ ms) were discarded. Finally, when *p*-values of interest were equal to this minimal *p*-value, the effect was considered significant only if its duration exceeded the longest duration observed at this *p*-value level within the baseline time-window. All statistics analyses were computed using Matlab 7.0 (Natick, MA, USA).

Individual regression analyses were computed with a similar method as in the group analysis. However, to perform a trial per trial statistic, we regressed each trial (congruent or incongruent) to the N400 and LPC templates. To avoid circular analysis for controls, templates were individually recomputed from the data of the 18 other subjects (e.g. "$n-1$ method"). We then compared, trial per trial, the beta values of congruent and incongruent trials using an unpaired *t*-test (see Fig. 4).

3. Results

We will first present group-level results both in controls and in DOC patients and then turn to individual statistics. Finally, diagnosis and outcome values of ERP effects will be reported for DOC patients.

3.1. Group-level analyses

3.1.1. Control subjects

The first significant ERP difference between incongruent and congruent trials was a N400 response lasting from 436 ms to 516 ms after the onset of second word (peaking at 472 ms, see Fig. 2). This N400 response was followed by a late and sustained posterior positive complex spanning from 652 ms to 1000 ms after the onset of the second word (peaking at 728 ms). This response shows the properties of the late positive complex (LPC) or P600 associated with the occurrence of semantic or syntactic incongruity (Pulvermüller et al., 2009).

We then estimated the cortical sources of these 2 ERP effects. The N400 effect was associated with an increase of cortical currents in the right temporal pole, in bilateral fronto-polar cortices with a right predominance, and left DLPFC (middle frontal gyrus), whereas the LPC corresponded to cortical activations maximum within left DLPFC (inferior frontal gyrus) and right fusiform gyrus (see Fig. 4).

3.1.2. DOC patients

We recorded 30 DOC patients (15 VS 15 MCS). One (MCS) patient has been excluded because of too many eye-blink artefacts. Fifteen patients were in vegetative state (VS), and fourteen were in the minimally conscious state (MCS) (see Table 2). We report the results of the DOC group as a whole, as well as results of the VS and MCS subgroups.

3.1.2.1. N400. The triple-threshold analysis revealed a significant effect within the N400 window in each of the 3 groups, spanning respectively from 280 to 394 ms for the DOC group, from 184 to 364 ms in the VS subgroup (peaking at 348 ms), and from 316 to 472 ms in the MCS subgroup (peaking at 360 ms).

Visual inspection of scalp topographies and the regression analysis to controls N400 template showed a partially preserved topography in the DOC group (marginally significant but long-lasting values in the regression statistics). Comparisons of the topographies of the VS and MCS subgroups with controls did not reveal any significant resemblance. Finally, we noted that the N400 occurred at a shorter latency than in controls (280–314 ms in DOC patients versus 436–516 ms in controls). No N400 effect was found when using the ROI approach. The N400 was more posterior in VS patients than in controls, and presented a right lateralization in MCS patients (see Fig. 3).

3.1.2.2. LPC. None of the three methods revealed any effect in the LPC window neither in DOC patients nor in VS patients. In sharp contrast, a strong LPC effect was observed in the MCS subgroup. Note that this effect had all the expected properties of the LPC: (1) it extended from 732 to 804 ms (peaking at 744 ms), a period included in the span of controls' LPC, (2) it showed a typical LPC topography both on visual inspection and with the regression analysis, and (3) it was significant in the left posterior ROI defined in controls, and close to significance in the right posterior ROI (see Fig. 3).

3.2. Individual-level analyses:

3.2.1. Controls

We probed effects of semantic congruence in individual subjects by looking for significant differences in the N400 and LPC windows using the usual triple-threshold, and also by running regression analyses to detect effects with expected topographies. A significant N400 effect was observed in 8/19 subjects (42.1%) with triple-threshold approach, and in 8/19 subjects (42.1%) with the regression analysis. Note that 5 subjects were positive with the two methods (see Fig. 5). We found a significant LPC in 6/19 subjects (31.6%; triple-threshold approach), and in 8/19 subjects (42.1%; regression approach). Three subjects were positive with the two methods. When requiring the presence of at least one of these two effects (N400 and/or LPC, using the triple-threshold approach), we were able to detect correlates of word-pair semantic processing in 11/19 (57.8%) of subjects. Note that we observed a large variability in latencies for both the N400 and the LPC (N400: 480[$\pm$ 63] ms; LPC: 853[$\pm$ 92]). There was also a large variability in topography: only 5 out of the 8 significant N400 as identified with the triple-threshold criteria had a significant N400 topography with the regression approach, and similarly only 3 out of the 6 LPC had a significant LPC topography.

3.2.2. Patients

A N400 effect was found in 6/29 DOC patients (5/14 MCS; 1/15 VS) with the triple-threshold approach. No additional patient could be identified using the regression approach (see Fig. 5). Therefore, the N400 component tended to be more present in MCS than in VS patients ($\chi^2=3.7$; $p=0.05$).

A LPC response was observed in 6 DOC patients (5/14 MCS; 1/15 VS) with the triple-threshold approach, and 3 additional MCS patients were positive using the regression approach. Overall, 8 MCS patients showed a LPC as compared with a single VS patient. The LPC was thus significantly more frequent in MCS than in VS patients ($\chi^2=8.6$; $p=0.003$). Three MCS patients showed both a N400 and a LPC response.

Given the variable number of blocks and of trials across patients and controls, we checked for the absence of relation between the presence/absence of N400 or LPC and the number of valid trials kept for EEG analysis after artefact rejection. For N400, observed values were of 285 $\pm$ 45 trials for 'N400+' and 256 $\pm$ 66 for 'N400−' in controls (*t*-test *p*-value=0.14), and of 304 $\pm$ 49 trials for 'N400+' and 293 $\pm$ 98 for 'N400−' in patients (*t*-test *p*-value=0.35). Similarly for LPC, observed values were of 261 $\pm$ 57 trials for 'LPC+' and 271 $\pm$ 61 for 'LPC−' in controls (*t*-test *p*-value=0.37), and of 278 $\pm$ 56 trials for 'LPC+' and 300 $\pm$ 97 for 'LPC−' in patients (*t*-test *p*-value=0.31).

3.2.3. Diagnostic and prognostic power of ERP effects

All patients were also recorded with the 'local–global' paradigm, which enables the detection of the low-level auditory P1 ERP component, of the automatic MMN response ('local effect') and of the ERP response to violations of the current auditory rule ('global effect'). As mentioned before, the latter is a very specific, – but weakly sensitive – , marker of conscious processing.

We now provide a full description of the diagnostic (being clinically diagnosed as VS or MCS) and prognostic (recovery of functional communication at 12 months, which also corresponds to being conscious and not in the vegetative or minimally conscious states) values of each of 5 ERP effects analysed in each patient: auditory P1, MMN, 'global effect', N400 and LPC. We decided to use a conservative statistical approach by considering only ERP effects passing the triple-threshold criterion (see Table 2, which also provides GOS-E outcomes). Prior to these analyses we noted that clinical diagnosis is a powerful predictor of functional communication outcome: whereas a positive outcome was observed for 7/14 (50%) MCS patients, only 1/15 (7%) VS patients recovered functional communication ($\chi^2=6.8$, $p=0.009$).

3.2.3.1. Auditory P1. Only 4/29 patients did not show early cortical P1 responses to sounds. Those 4 patients were in a VS during ERP recording (3 from anoxic lesions and 1 from severe traumatic brain injury). The absence of P1, in the absence of severe dysfunction of the peripheral or central auditory pathway was systematically associated with VS (Sensibility (Se)=100%; Specificity (Sp)=27%; Predictive Positive Value of being MCS (PPV)=56%; Negative Predictive Value of being MCS (NPV)=100%). Therefore, the P1 showed an ideal sensitivity for MCS, and its absence perfectly predicted the VS. Given this strong association with VS, analyses of the prognostic value of this ERP component is essentially confounded with the initial clinical status (Se=100%; Sp=19%; PPV=32%; NPV=100%).

3.2.3.2. MMN (or local effect)

19/29 patients had a significant MMN, including 12/14 MCS patients and 7/15 VS patients (Se=86%; Sp=53%; PPV=63%; NPV=80%). These values resemble to those observe for the P1 ERP component. MMN showed a similar sensitivity for prognosis and a strong NPV (Se=88%; Sp=43%; PPV=37%; NPV=90%).

3.2.3.3. N400. 6/29 patients presented a significant N400 response. All but one (5/6) were in a MCS (Se=36%; Sp=93%;

Table 2
Patients' characteristics, ERP results and outcomes.

State	Age	Sex	CRS sub-scores[a]						CRS	Etiology	Delay (days)[b]	P1/ N1	Local Effect	Global Effect	N400	LPC	Language outcome [c]	Consciousness recovery	GOS-E 6 months	GOS-E 12 months
			1	2	3	4	5	6	Total											
MCS-1	56	Male	1	3	0	1	0	1	6	Other	134	+					0		2	1
MCS-2	59	Female	2	0	3	3	1	0	9	Other	60	+					0		1	1
MCS-3	34	Male	2	3	4	1	0	2	12	TBI	26	+	+				2	+	4	6
MCS-4	33	Female	1	3	2	2	0	2	10	Stroke (c)	10	+	+				1	+	3	4
MCS-5	24	Male	1	3	2	2	0	2	10	TBI	1259	+	+				0		3	3
MCS-6	43	Female	1	3	2	1	0	2	9	Stroke	25	+	+			+	0		2	1
MCS-7	47	Male	3	3	3	2	0	1	12	TBI	1583	+	+		+		2	+	3	3
MCS-8	54	Male	2	3	2	0	0	2	9	Stroke (c & b)	60	+	+		+		0		1	1
MCS-9	64	Male	3	0	2	2	1	2	10	Stroke (b)/other	30	+	+		+	+	2	+	3	3
MCS-10	28	Male	1	3	0	1	0	1	6	TBI	17	+	+	+			2	+	4	7
MCS-11	18	Male	1	3	3	1	0	2	10	TBI	33	+	+	+			2	+	7	7
MCS-12	58	Male	3	3	2	2	1	2	13	Anoxia	60	+	+	+		+	2	+	1	1
MCS-13	23	Male	2	3	0	1	0	2	8	Other	87	+	+	+	+	+	1		3	3
MCS-14	36	Male	1	1	0	1	1	2	6	Stroke (b)	24	+	+	+	+	+	2	+	3	3
VS-1	59	Male	1	0	0	1	0	1	3	TBI	30						0		1	1
VS-2	29	Female	0	0	1	1	0	2	4	Anoxia	26						1		3	3
VS-3	78	Male	0	0	1	1	0	2	4	Anoxia	15						0		1	1
VS-4	52	Male	1	0	1	1	0	1	4	Anoxia	23						0		1	1
VS-5	22	Female	1	0	2	1	0	2	5	Anoxia	30	+					0		2	2
VS-6	48	Male	0	1	1	2	0	2	6	Stroke (c)	497	+					0		2	1
VS-7	59	Female	1	1	2	1	0	1	6	Stroke (c)	21	+			+		2	+	4	4
VS-8	46	Male	1	0	1	1	0	2	5	Anoxia	155	+					0		2	2
VS-9	31	Male	1	0	0	1	0	1	3	Anoxia	7	+	+				0		2	1
VS-10	37	Female	1	0	2	0	0	1	4	Stroke (c)	194	+	+				0		2	2
VS-11	37	Female	1	1	1	1	0	1	5	Stroke (c)	62	+	+				1		3	3
VS-12	48	Male	0	0	2	1	0	2	5	Anoxia	15	+	+				0		1	1
VS-13	40	Male	1	1	2	1	0	1	6	TBI	62	+	+				0		1	1
VS-14	62	Male	0	0	0	0	0	1	1	Other	30	+	+			+	0		1	1
VS-15	64	Female	1	1	2	1	0	2	7	Other	23	+	+				0		2	2

CRS-R = Coma Recovery Scale-Revised.

[a] CRS-R subscores incude: 1: auditory function; 2: visual function; 3: motor function; 4: oromotor/verbal function; 5: communication scale; 6: arousal scale.

[b] Delay from acute brain injury (in days); TBI = traumatic brain injury; VS = vegetative state; MCS = minimally conscious state; Etiology column: stroke could affect cortical (c), or brainstem structures (b).

[c] Language outcome = best CRS--sub-score-5 after ERPs recording. GOS-E = Glasgow Outcome Scale-Extended.

PPV = 83%; NPV = 61%). Thus, the N400 showed an interesting capacity to differentiate MCS from VS patients, as well as a valuable positive predictive value for the MCS. Note that the etiologies of patients showing a N400 response included stroke and TBI, but not anoxia. In terms of prognostic value, the presence of a N400 component was a specific marker of communication recovery, endowed with a strong negative predictive value (Se = 50%; Sp = 90%; PPV = 67%; NPV = 83%). Interestingly, the single VS stroke patient with a N400 response recovered consciousness and functional communication.

3.2.3.4. LPC. 6/29 patients presented a significant LPC response, only one of which suffering from anoxia. All but one (5/6) were in a MCS (Se = 36%; Sp = 93%; PPV = 83%; NPV = 61%). Among those 6 patients, 3 also showed a N400 response. Note that two of them recovered consciousness and functional communication, whereas the third remained in a MCS. In terms of prognosis, only 3 of the LPC+ patients recovered a functional communication (Se = 38%; Sp = 86%; PPV = 50%; NPV = 78%). The single VS patient with a LPC response did not recover functional communication.

3.2.3.5. Global effect. 5/29 patients presented a significant 'global effect', only one of which suffering from anoxia. All patients showing a 'global effect' were in a MCS (Se = 36%; Sp = 100%; PPV = 100%; NPV = 62%), as we previously described in a larger series (Faugeras et al., 2012). In terms of prognostic value, presence of a 'global effect' component was a specific marker of communication recovery, endowed with a strong negative predictive value (Se = 50%; Sp = 95%; PPV = 80%; NPV = 83%).

4. Discussion

In the present study we tried to develop an electrophysiological index of semantic processing which could be used to refine the diagnosis and prognosis of patients with disorders of consciousness in addition to clinical and behavioral examination. To this end, we recorded two ERP markers (N400 and LPC) elicited by pairs of semantically congruent auditory words, as compared to incongruent words. Our results are twofold: First, we provide novel findings regarding the spatio-temporal cortical dynamics of verbal semantic processing in conscious subjects. Second, we determined the medical value and shortcomings of those markers in vegetative and minimally conscious patients. Those two points will be discussed in turn.

4.1. Verbal semantic processing in conscious healthy subjects

The earliest significant difference between congruent and incongruent word pairs was the classical N400 component,

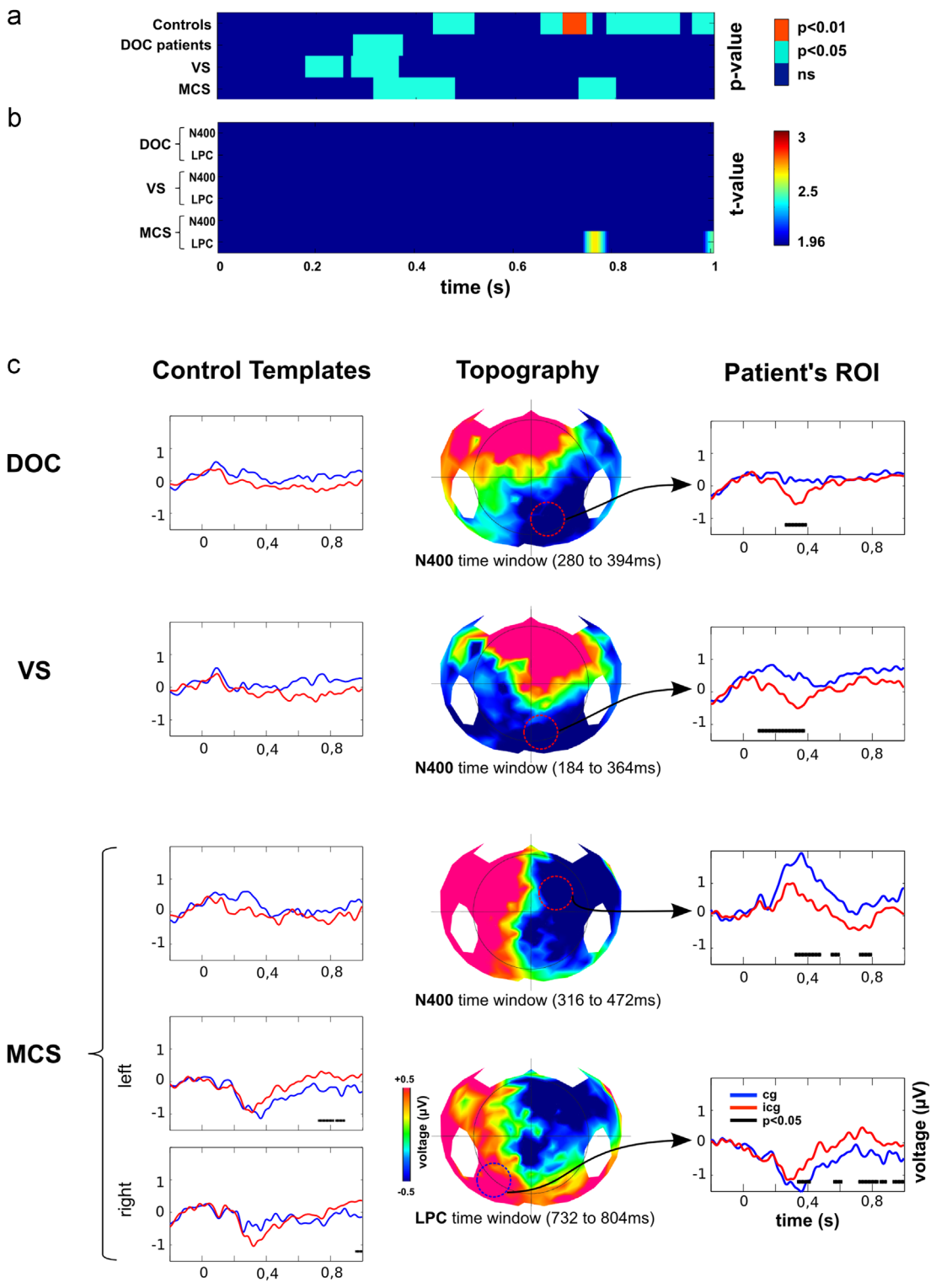

Fig. 3. N400 and LPC in DOC patients (a) overview of semantic congruity effects analysed with the triple-threshold method in: controls (upper line, see Fig. 2a), DOC patients (second line), MCS patients (3rd line) and VS patients (4th line) groups. A N400 ERP was detected in the 3 groups of patients, whereas the LPC was only found in the MCS group. (b) Overview of semantic congruity effects analysed with the regression approach using controls N400 and LPC templates in: DOC patients (1st & 2nd lines), in MCS (3rd & 4th lines) and in the VS (5th & 6th lines) groups. This analysis confirmed that the LPC detected in the MCS patients group with the triple-threshold method presented a significant normal LPC topography. (c) Patients' groups ERPs and voltage topographies in N400 and LPC ROIs defined with the controls group.

spanning from ~400 ms to ~550 ms after the onset of the second word. We did not find any earlier ERP response to semantic congruity, such as the early left anterior negativity (ELAN) (Friederici and Mecklinger, 1996). This negative finding may have several explanations. First, the ELAN may actually be a marker of early syntactic processing, whose absence is congruent with the absence of any syntactic structure in our stimuli (Friederici, 2004). Alternatively, it cannot be excluded that, due to the size of our sample, we lacked sufficient statistical power to detect this early marker.

In agreement with previous reports (Kutas and Federmeier, 2011), the N400 consisted in a centro-parietal negativity with

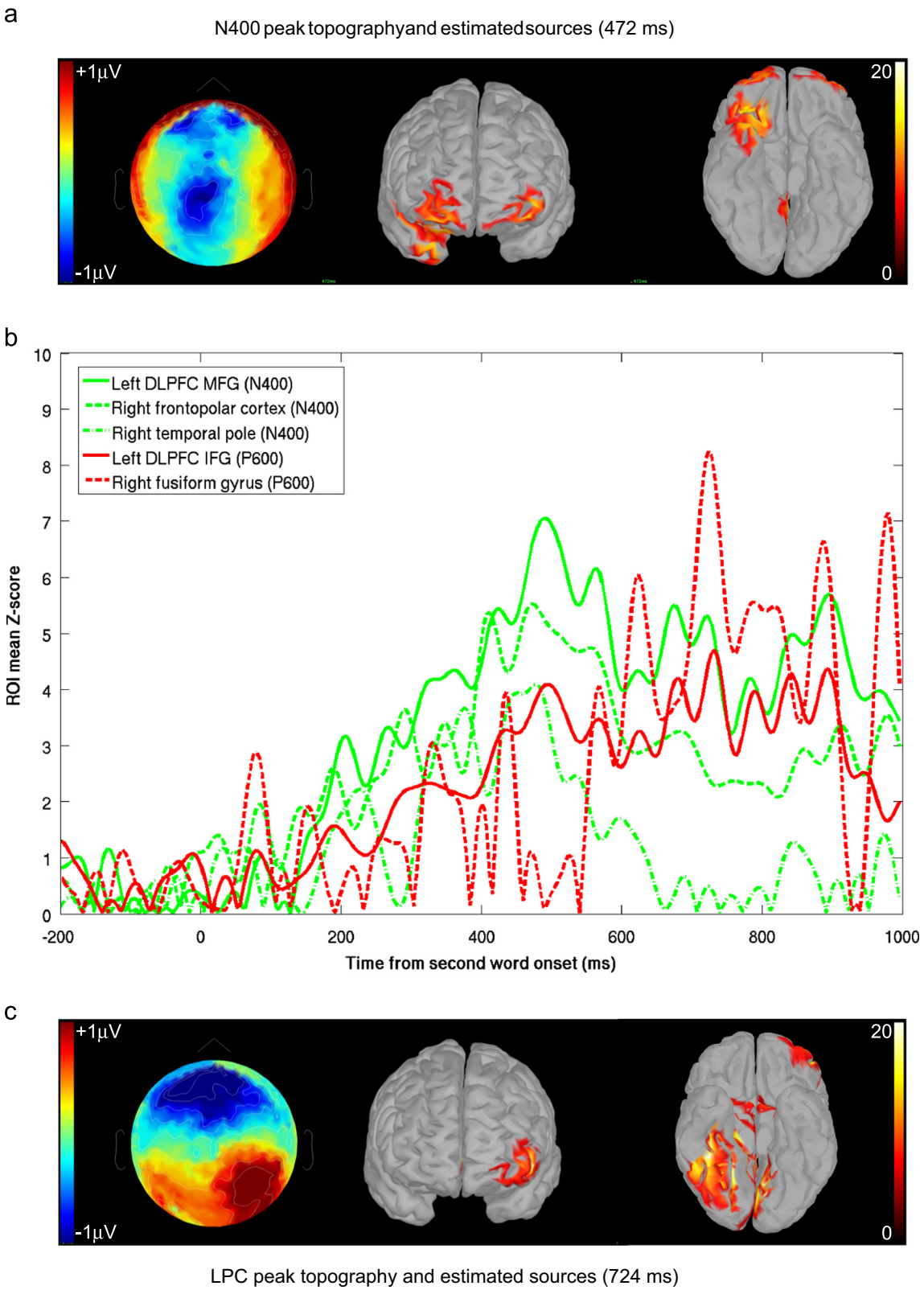

Fig. 4. Cortical sources of N400 and LPC in controls (a) N400 estimated cortical sources at the peak of the effect (472 ms). (b) Time-courses of *Z*-scores (computed in reference to a 200 ms baseline) respectively for N400 sources in green (left DLPFC MFG, right frontopolar cortex and right temporal pole), and for LPC sources in red (left DLPFC IFG and right fusiform gyrus) and (c) LPC estimated cortical sources at the peak of the effect (724 ms).

moderate left-lateralization (see Fig. 2). Its estimated sources were located in frontal and temporal structures, in particular in the right fronto-polar cortex, in the left DLPFC (middle frontal gyrus) and in the right temporal pole (see Fig. 4). This pattern matches the seminal description of the N400 time-course. As reviewed by Kutas and Federmeier (2011) the N400 usually corresponds to: "*a wave of activity starting at* 250 ms *in the posterior half of the left superior temporal gyrus, spreading first forward and ventrally to the*

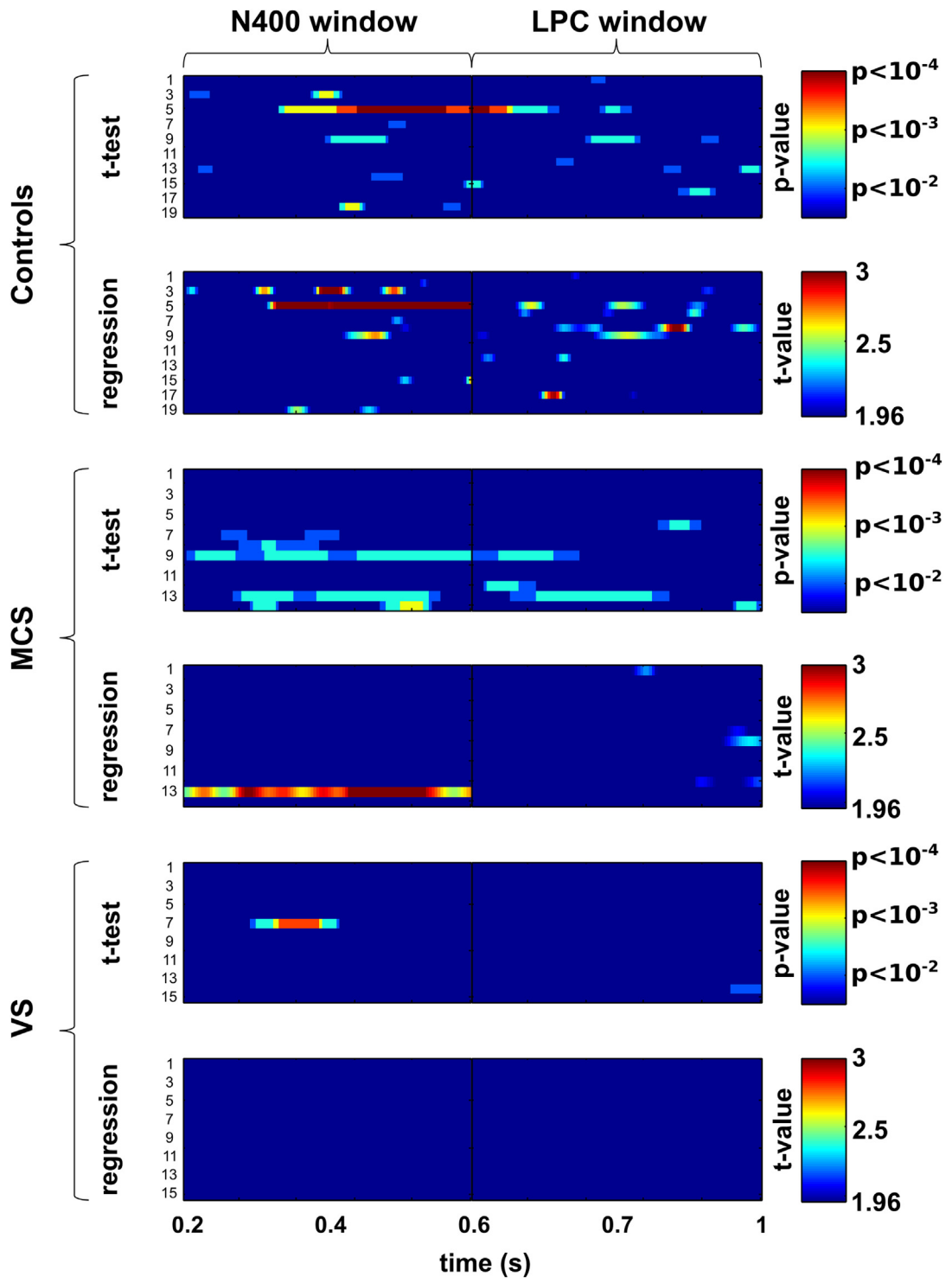

Fig. 5. Individual ERP results of semantic congruity in controls and in patients Each line corresponds to one subject (one of the 19 controls, 14 MCS patients or 15 VS patients). Congruity effects significant at the individual level are color-coded, both for the triple-threshold ("*t*-test") and the regression ("regression") approaches.

left temporal lobe by 365 *ms, and thereafter, between* 370 *and* 500 ms, *to the right anterior temporal lobe and to both frontal lobes*". When we inspected the estimated sources of the N400 we also observed an early activation in the left inferior temporal region, a region involved in supramodal word processing (Cohen et al., 2004; Price, 2012). This source was activated earlier than the significant N400 effect. Hence one may wonder whether the absence of syntactic structure of our stimuli suppressed both early syntactic processes (ELAN) and early component of the N400 which could be related to syntactic or lexico-syntactic processes. Under this view, our finding could be interpreted as a more specific correlate of lexico-semantic processing, independently of other parameters such as those implicated by the existence of a syntactic structure. This hypothesis would deserve additional studies using more reliable functional brain-imaging techniques, such as fMRI or SEEG recordings. In addition, it is interesting to

note that previous studies underlined the importance of right-hemisphere temporal and frontal structures in semantic processing, in particular when complex sentences, metaphors or idioms were used (St. George et al., 1999). These authors reported fMRI activations within the right temporal lobe in response to titled paragraphs as compared with untitled paragraphs, suggesting a specific role of this region in global semantic processing.

The second correlate of semantic congruity was the LPC (or P600), which appeared as a late and sustained posterior positive complex spanning from ~700 ms to 1000 ms after the onset of the second word. Its estimated sources were mostly located in the left DLPFC (inferior frontal gyrus) and the right fusiform gyrus (see Fig. 4). While early studies described this ERP component as a marker of syntactic violation (Friederici and Meyer, 2004), recent studies challenged this interpretation by showing LPC in response to semantic violations or anomalies in the absence of any syntactic violation (Grieder et al., 2012; Hill et al., 2002). In addition, a LPC could be recorded in response to various manipulations of verbal semantics such as inversion of causality (e.g. "*the cat that fled from the mice*", (Van Herten et al., 2005)), metaphors (De Grauwe et al., 2010) or ironic stimuli (Regel et al., 2011; Spotorno et al., 2013). We propose that the LPC (or P600) could reflect conscious access to the semantic violation, whereas the N400 would reflect an earlier and non-conscious stage of semantic processing. This hypothesis is supported by 2 main arguments. First, many studies using both auditory and visual stimuli support a two-stage model of word perception, with an early non-conscious stage followed by a later stage requiring a conscious access (Gaillard et al., 2009; Marinkovic et al., 2003). While the early stage is usually restricted to localized brain networks (e.g.: MMN, early visual processing), the late stage is associated with a brain-scale increase in functional connectivity. This two-stage hypothesis is part of several models of conscious access including the global workspace model of consciousness (Dehaene et al., 2006; Dehaene and Naccache, 2001; Lamme, 2006; Sergent and Naccache, 2012). The second argument supporting our hypothesis concerns the LPC component itself. While the latency and topography of the N400 are very different from those of earlier stages of perceptual processing (e.g. the right fusiform N170 for faces; the left fusiform N200 for printed words; the MMN for deviant sounds in odd-ball paradigms), the LPC shares a latency (~200 ms after first-stage processing) and a scalp-topography reminiscent of the P3b. Given that this P3b event has been described as a specific marker of conscious access to visual stimuli (Sergent et al., 2005). This similarity between the LPC and the P3b would therefore suggest that the LPC is a neural marker of conscious semantic processing.

Interestingly, this theoretical interpretation is supported by previous works originating from memory studies. Indeed, the LPC component has been associated with conscious recollection of words (Düzel et al., 1997; Petten et al., 1991). For instance, (Düzel et al., 1997) used a "remember/know" paradigm and reported that the LPC was present exclusively for consciously remembered words. In 2003, (Misra and Holcomb, 2003) used a repetition priming paradigm with both masked and unmasked prime words preceding unmasked target words. While the N400 was attenuated both for the masked and unmasked repetition priming conditions, a significant increase of the LPC component was exclusively observed in the unmasked repetition priming condition. Note also that if the LPC observed in our work was related to conscious recollection, this would still require a semantic stage in order to explain why incongruent pairs would be better memorized than congruent ones. In order to better assess this issue, we analysed separately the LPC in first and second experimental blocks. Using our triple-threshold procedure, a significant LPC was observed both in the first and in the second blocks (both $ps < 0.05$).

In spite of their impact on our fundamental understanding of verbal semantic processing, our findings were not as powerful as we could expect, which may limit their clinical applications. Only 11/19 (58%) conscious controls showed a significant effect within the respective temporal windows of the N400 or of the LPC. In contrast, we could identify a significant P3b component in response to violations of auditory regularities in 100% of control subjects tested in the "local–global" test (Bekinschtein et al., 2009; Faugeras et al., 2012; King et al., 2013). This lack of sensitivity at the individual level may reflect a large inter-trial variability in the precise timing of semantic processing. While our results are in line with robust research requirements at the group-level by finding traditional effects in controls, the absence of systematic detection of N400 or LPC effects at the individual-level is problematic, in particular for a translational goal aiming at using these measures to improve patients' evaluation. Even within control subjects, we observed a large variability in the topography of the N400 (only 5/8 subjects with a typical N400 topography), and of the LPC (3/9 subjects with a typical LPC topography). In order to boost the N400 and LPC, we could use active tasks focused on semantic attributes of word pairs, rather than instructing subjects to simply attend to the stimuli without engaging in any specific task. Indeed, (Cruse et al., 2014) designed three experiments in normal controls, and showed that while N400 could be detected at the single-subject level in a semantically related word-pair paradigm using an active response task in 75% (9/12) of subjects, this proportion dropped to 58% (7/12) when subjects were instructed to simply pay attention to the semantic relation covertly, and to 0% when they were asked to passively listen to the material. Then in a second experiment, they showed that the use of word-pair stimuli generated from normative associations – as done in our work – increased the detection of N400 in the passive condition in 50% of subjects. Interestingly, the use of cloze sentences proved to be less effective (17%) than word pairs, which is not intuitive given the stronger semantic expectations elicited by a sentence induced context than by a single word. Indeed, verbal semantic brain activations has been reported with PET and fMRI in a few patients using sentences (see Section 1.2). Clearly, this issue of word-pairs versus sentence paradigms still requires additional studies because many factors distinguish these two paradigms, and their respective impact are not necessarily easy to predict. For instance, working memory and language impairments in patients may explain both patterns of differential results: on the one hand, sentences could be processed less efficiently than word-pairs, but alternatively, the cumulative added contextual "strength" provided by a sentence might be sufficient to overcome this deficit, and induce efficient top-down semantic processes. Our current results in controls are close the ones of this second experiment of Cruse et al.

This importance of using an active task is also coherent with our own previous results with the "local global" task. Indeed, the P3b response to the auditory rule violation ("local global" test) can be observed in a passive condition comparable to the one we used in the present study, but is considerably enhanced when subjects are asked to detect and count rule violations (Bekinschtein et al., 2009; King et al., 2013; Wacongne et al., 2011). This finding has been reliably observed in many "active paradigms", in particular when testing patients suffering from disorders of consciousness (Owen et al., 2009; Schnakers et al., 2008). Additionally, it is possible that the use of more complex and meaningful stimuli containing also syntactic structures, – such as short sentences or idioms – , may be more efficient.

4.2. Verbal semantic processing in non-communicating patients

At the group-level, we could detect a significant N400-like response in DOC patients. This ERP response peaked earlier than

the N400 observed in conscious controls (280–314 ms in DOC patients versus 436–516 ms in controls), and its topography resembled only partially the normal N400. Indeed, whereas a clear central negativity was present in DOC patients, both the ROI and the regression analyses showed only marginal values of similarity with the canonical N400. Concerning the earlier latency of patients' N400, we note that across the 6 previous studies conducted in DOC patients one found a discretely delayed (<20 ms) latency of N400 for incongruent trials (Balconi et al., 2013), whereas no specific information was reported in the other 5 articles (Kotchoubey, 2005; Kotchoubey, et al., 2005; Rämä et al., 2010; Schoenle and Witzke, 2004; Steppacher et al., 2013). Larger series of patients with homogenous aetiologies would be necessary to establish reliable landmarks on this issue, as it is plausible that different aetiologies should be associated to different patterns of impairments of the brain's semantic networks. Concerning the topography of the N400 in the DOC group, – and in the VS and MCS subgroups – , we mostly retain its overall similarity with the normal pattern, and do not emphasize the left-right asymmetry observed in the MCS group. Larger and more homogeneous samples of patients seem necessary to establish a stable picture of the results. The result which most deserves to be highlighted is the confirmation that a N400 effect can be observed in vegetative state patients.

In sharp contrast with the N400, the LPC was absent in the VS patients group. This is remarkable because the LPC was observed in the MCS group with a latency and a topography highly similar to those observed in the group of controls. This result strongly supports our proposal that the LPC may be a specific marker of conscious access to semantic attributes. It is also coherent with our previous finding that only conscious and MCS patients showed a late P3b response indexing the conscious detection of violations of a long-range auditory rule (Bekinschtein et al., 2009; Faugeras et al., 2012; King et al., 2013). As previously mentioned, many studies conducted in the visual modality extended the validity of this two-stage model of conscious perception (Gaillard et al., 2009; Sergent et al., 2005). Moreover, our finding of a significant LPC in MCS patients further confirm the relevance of this recently defined clinical category (Giacino et al., 2002; Kalmar and Giacino, 2005). Indeed, if the LPC is a signature of the active maintenance of information in a brain-scale coherent network corresponding to conscious perception, then its presence in MCS patients would confirm the presence of conscious states in those patients. This claim is also supported by our recent report that MCS patients present, – like conscious patients – significant long-range functional connectivity, as measured with a new mathematical measure (weighted Symbolic Mutual Information, wSMI), whereas this property is massively impaired in VS patients (King et al., 2013).

At the individual level, our results were restricted by the limited sensitivity of the N400 and LPC to verbal semantic processing in controls. The N400 was mostly detected in MCS, while only a single VS patient (who later recovered consciousness and functional communication) showed this response. This finding corroborates previous reports showing a more reliable N400 in MCS than in VS (Schoenle and Witzke, 2004; Steppacher et al., 2013). We found a similar pattern for the LPC component. However, the comparison of both group-level and individual results lead us to propose a distinct explanation for the similarity of individual results of these two ERP components. In the light of our theoretical distinction between the two successive stages of perception, the extreme rarity of LPC in VS patients may reflect a qualitative difference between VS and MCS patients dependent on the presence/absence of a conscious access to a semantic representation. In contrast, the similarly low proportion of N400 in VS patients as compared to MCS patients may rather reflect a quantitative difference: the early stage of unconscious semantic processing may be weaker but still present in some VS patients.

Finally, we could compare the diagnostic and prognostic value of five distinct ERP responses. The inspection of these 5 ERP correlates is suggestive of a “Jacksonian” hierarchical organization, from low-level automatic events (P1 and MMN) to high-level cognitive processes (N400, LPC and global effect) more related to functional communication and to conscious processing. Indeed, the two late ERP components (the global effect and the LPC) were systematically observed in patients also showing preserved P1 and MMN responses, while many patients showed only the early or automatic responses. This was also true for the N400 responses, which were systematically observed in patients with preserved P1 and MMN responses. However, the relative dissociation between N400, LPC and global effect may reflect a diversity of high-level cognitive processors, which may operate independently.

In conclusion, we note that the LPC observed in controls and in MCS groups shares many properties of the P3b responses reported in other auditory or visual paradigms during conscious access to perceptual representations. On the basis of these findings and the two-stage model of perception, we proposed to identify the LPC with a conscious stage of semantic processing of verbal stimuli.

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Appendix A. Supplementary Information

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