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Progression of auditory discrimination based on neural decoding predicts awakening from coma

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Auditory evoked potentials are informative of intact cortical functions of comatose patients. The integrity of auditory functions evaluated using mismatch negativity paradigms has been associated with their chances of survival. However, because auditory discrimination is assessed at various delays after coma onset, it is still unclear whether this impairment depends on the time of the recording. We hypothesized that impairment in auditory discrimination capabilities is indicative of coma progression, rather than of the comatose state itself and that rudimentary auditory discrimination remains intact during acute stages of coma. We studied 30 post-anoxic comatose patients resuscitated from cardiac arrest and five healthy, age-matched controls. Using a mismatch negativity paradigm, we performed two electroencephalography recordings with a standard 19-channel clinical montage: the first within 24 h after coma onset and under mild therapeutic hypothermia, and the second after 1 day and under normothermic conditions. We analysed electroencephalography responses based on a multivariate decoding algorithm that automatically quantifies neural discrimination at the single patient level. Results showed high average decoding accuracy in discriminating sounds both for control subjects and comatose patients. Importantly, accurate decoding was largely independent of patients' chance of survival. However, the progression of auditory discrimination between the first and second recordings was informative of a patient's chance of survival. A deterioration of auditory discrimination was observed in all non-survivors (equivalent to 100% positive predictive value for survivors). We show, for the first time, evidence of intact auditory processing even in comatose patients who do not survive and that progression of sound discrimination over time is informative of a patient's chance of survival. Tracking auditory discrimination in comatose patients could provide new insight to the chance of awakening in a quantitative and automatic fashion during early stages of coma.

Introduction

Impairment in auditory functions has been repeatedly reported in comatose patients (Kane *et al.*, 1993; Fischer *et al.*, 1999, 2004; Daltrozzo *et al.*, 2009) and patients in a minimally conscious or vegetative state (Neumann and Kotchoubey, 2004; Kotchoubey *et al.*, 2005; Boly *et al.*, 2011). Typically, these clinical populations show deficits in neural discrimination between repeated (standard) and rare (deviant) sounds assessed during a mismatch negativity paradigm (Näätänen *et al.*, 1978). Mismatch negativity is described as an automatic fronto-central Electroencephalogrphy (EEG) component, occurring at ~100–150 ms after the onset of deviation (Garrido *et al.*, 2009). Interestingly, mismatch negativity appears to correlate with the depth and severity of consciousness disorders in patients in a vegetative or minimally conscious state (Boly *et al.*, 2011) and to be absent in those comatose patients who do not awake from the coma (Fischer *et al.*, 2004). Therefore, the presence of the mismatch negativity is considered to be a predictor of awakening, with high predictive value for awakening (Kane *et al.*, 1993; Fischer *et al.*, 2004; Naccache *et al.*, 2005; Wijnen *et al.* 2007).

However, because in these studies, mismatch negativity is assessed at various delays after coma onset (e.g. 10.34 ± 11.4 days in Fischer *et al.*, 2004) or several months after the onset of consciousness impairment in patients in a vegetative state (Bekinschtein *et al.*, 2009; Boly *et al.*, 2011), it is still unclear whether the mismatch negativity deficit is independent of the time of the recording. Moreover, previous studies testing mismatch negativity in post-anoxic comatose patients were performed in normothermic conditions. Today, however, mild induced therapeutic hypothermia has become a standard of care in this setting (Oddo *et al.*, 2006). Therapeutic hypothermia is known to have neuroprotective effects on the patients and to increase their chance of survival (Holzer, 2010), but its effect on brain functions remains unknown. Here, we hypothesized that the absence of mismatch negativity could be the result of the degeneration of auditory functions over time and that during the very early phase of coma and under therapeutic hypothermia, auditory processing might still be intact. We therefore focused on the acute phase of coma in a homogeneous cohort of patients after hypoxic–ischaemic encephalopathy, all of whom were treated with mild induced therapeutic hypothermia, according to a standardized procedure (Oddo *et al.*, 2006; Rossetti *et al.*, 2010).

Typically, the presence of mismatch negativity is assessed by averaging thousands of single-trial EEG responses to standard and deviant sounds and then subtracting one type of response from the other, for one electrode or a group of electrodes (Fischer *et al.*, 1999; Wijnen *et al.*, 2007; Todd *et al.*, 2008). According to this approach, mismatch negativity evaluation requires the identification of a robust auditory evoked potential response to sounds (i.e. a significant modulation of an N100 component). Therefore, data from a large percentage of patients are systematically disregarded (e.g. ~33% in Fischer *et al.*, 1999). Furthermore, this assessment requires an *a priori* hypothesis of the latency and the magnitude of auditory evoked potential responses. In pathological conditions, making such hypotheses can be challenging, as auditory evoked potentials can exhibit high inter-individual variability and differ from those of controls.

To address these issues, we quantified the degree of differences of the neural responses to standard versus deviant sounds using a multivariate EEG decoding approach that takes advantage of the distribution of voltage measurement across the whole electrode montage (De Lucia *et al.*, 2007, 2010; Tzovara *et al.*, 2012a, 2012c). This method allows the separate analysis of neural responses for each patient, without preliminary assessment of minimal inclusion criteria. Decoding of EEG responses to standard versus deviant sounds was computed twice per patient, once during the first 24 h of coma and under therapeutic hypothermia and a second time, ~24 h later, after rewarming to normothermic conditions (Table 1). The improvement of this decoding accuracy is informative of the progression of auditory functions during the acute stage of coma.

Materials and methods

Patients and controls

We included 30 post-anoxic comatose patients (10 females; mean age $\pm$ SEM, 63 ± 2 years) admitted from December 2009 to July 2011 to the Department of Critical Care Medicine, Centre Hospitalier Universitaire Vaudois (CHUV-Lausanne University Hospital), Lausanne, Switzerland. All patients were treated with mild therapeutic hypothermia after resuscitation from cardiac arrest, to 33°C for 24 h. The study was approved by the Ethics Committee of the institution.

Level of consciousness was assessed based on the Glasgow Coma Scale at regular intervals (every 2–3 h) during the first 48 h after coma onset. All patients scored 3 or 4 during these first 48 h, indicating unconscious state.

All patients were managed according to a standard protocol (Oddo *et al.*, 2006); they were resuscitated following current recommendations (American Heart Association, 2005) and treated with mild therapeutic hypothermia to 33°C for 24 h, using ice-packs, intravenous ice-cold fluids and a surface cooling device (Arctic Sun System, Medivance) for the maintenance of therapeutic hypothermia, during which midazolam (0.1 mg/kg/h) and fentanyl (1.5 µg/kg/h) were administered for sedation and vecuronium (0.1 mg/kg boluses) to control shivering.

Patients with myoclonus and/or status epilepticus were treated with intravenous anti-epileptic drugs, which were discontinued if no clinical improvement was noted after at least 72 h. An interdisciplinary decision on withdrawal of intensive care support was based on a multimodal approach (Rossetti *et al.*, 2010) including at least two of the following (assessed in normothermia at least 48–72 h after cardiac arrest): incomplete recovery of brainstem reflexes, early myoclonus and bilaterally absent cortical somatosensory evoked potentials. Specifically, the results of the present study were not used for this decision. Patients' clinical outcome at 3 months was categorized as awake or dead. After 3 months from coma onset no patients remained in a vegetative state.

Among the 30 patients, the first 12 admitted to the hospital formed a pilot group and the rest a validation group. We analysed data from the pilot group in a more exploratory manner and validated our results with the validation group (18 patients). All analysis in the validation group was done blindly to the patients' outcome.

Table 1 Patient characteristics

	Decoding improves	Decoding drops	P-value
Patients alive at 3 months, %	100	45	
Patients deceased at 3 months, %	0	55	
Age, years	59 ± 5	63 ± 3	0.58
Time to return of spontaneous circulation, min	17 ± 3	22 ± 3	0.40
Time to first EEG, h	15 ± 2	15 ± 1	0.74
Time between recordings, h	23 ± 1	34 ± 4	0.08

Description of the patients, separated according to the evolution of decoding performance from the first to second recording (second and third columns, respectively). We provide details about the patients' mean age, duration of cardiac arrest (i.e. the time from circulatory arrest to return of spontaneous circulation), time from cardiac arrest to the first EEG recording, time between recordings, and the proportion of patients who awoke from coma and were alive at 3 months. We performed statistical comparisons for the first four of these variables, but none differed significantly between patients whose decoding performance improves and those whose decoding performance drops from first to second recording (Mann–Whitney U-test). Values are mean $\pm$ SEM.

We additionally considered five control subjects in the same age range (three females; mean age 54 ± 2 years). None had a history of neurological or psychiatric illnesses, and all reported normal hearing. We carried out the same experiment and data analysis on controls. The goal was to evaluate the level of auditory discrimination that could be obtained using our approach at the single-subject level in a healthy population. However, at no point did we perform a quantitative comparison between auditory discrimination of patients and controls (for which a higher number of controls would be required).

Stimuli

We employed an auditory mismatch negativity paradigm involving one standard and three types of deviant sounds presented to the patients and control subjects via earphones with a constant interstimulus interval of 700 ms. Standard sounds occurred in 70% of the trials and consisted of 1000 Hz sinusoidal tones of 100 ms duration and 0 μs inter-aural time difference. The pitch deviants (10% of trials) were 1200 Hz sinusoidal tones of 100 ms duration and 0 μs inter-aural time difference. The duration deviants (10% of trials) were 1000 Hz sinusoidal tone of 150 ms duration and 0 μs inter-aural time difference. Finally, the location deviants (10% of trials) were 1000 Hz sinusoidal tones of 100 ms duration and 700 μs inter-aural time difference (left ear leading). A 10-ms linear amplitude envelope at stimulus onset and offset was applied to all stimuli to avoid clicks. All stimuli were 16-bit stereo sounds sampled at 44.1 kHz. These properties were in accordance with other mismatch negativity studies (Todd et al., 2008). A block of trials included 500 stimuli and lasted ~7 min. Stimuli were presented in a pseudo-randomized order, such that at least one standard stimulus intervened between deviants. We recorded three blocks, resulting in 1500 trials per participant (1050 for the standard sound and 150 per each deviant sound). In order to keep the groups of sounds balanced and to also ensure habituation in the processing of standard sounds, for all analyses we only considered responses to the standard sounds that occurred after another standard sound and that were preceding a deviant one.

Electroencephalography acquisition and preprocessing

Continuous EEG (Viasys Neurocare) was recorded during therapeutic hypothermia and normothermic paradigms, with 19 electrodes arranged following the international 10–20 system. We used a sampling rate of 1024 Hz with an online reference to the Fpz electrode.

All electrodes' impedances were kept below 10 kΩ. All EEG recordings were performed in the intensive care unit, while patients were lying on their beds, without interrupting the clinical routine. For reasons of consistency, control recordings on healthy subjects were performed with the same set-up and equipment, in a hospital room, while they were lying on an inclined chair. Controls were instructed to close their eyes and listen to the sounds.

EEG preprocessing was performed using Cartool (Brunet et al., 2011). Peri-stimulus epochs were extracted, spanning 50 ms before the sound onset, up to 500 ms post-stimulus onset. An artefact rejection criterion of ± 100 μV was applied offline at all 19 electrodes. Data from noisy electrodes were interpolated using 3D splines (Perrin et al., 1987). Data were re-referenced offline, to the common average reference and were 0.1–40 Hz band-pass filtered. No prestimulus baseline correction was applied [see Michel et al. (2009) for discussion on baseline correction].

Multivariate decoding

For quantifying the difference in neural responses to standard versus deviant sounds, we used a multivariate EEG analysis (Tzovara et al., 2012a, 2012c). The suitability of this method for analysing EEG data has been demonstrated in several studies (Bernasconi et al., 2011; De Lucia et al., 2012; Tzovara et al., 2012b). One advantage of using this multivariate technique is that it is not biased by a priori hypotheses about electrode location(s) at which stimulus-related activity is expected. Therefore, it is less affected by transient artefact-contaminated activity appearing at some specific electrodes than classical analyses of single-electrode average auditory evoked potentials. In addition, it provides a way to quantify differences in neural responses at the level of the single patient, without preliminary assessment of minimal inclusion criteria.

This method is based on modelling the voltage topographies of the single-trial auditory evoked potentials by a Mixture of Gaussians. This analysis was performed separately for each patient and for each of the two recording data sets (i.e. under therapeutic hypothermia and under normothermic conditions). Mixture of Gaussians estimation was based on part of the available trials (training data set) and was then used to decode the category of sounds (standard/deviant) on a separate part of the data set (test data set; see Supplementary material). Decoding performance is indicative of the degree of difference in single-trial brain responses to standard versus deviant sounds. Importantly, because the analysis is based on voltage topographies, an accurate performance is a direct result of the activation
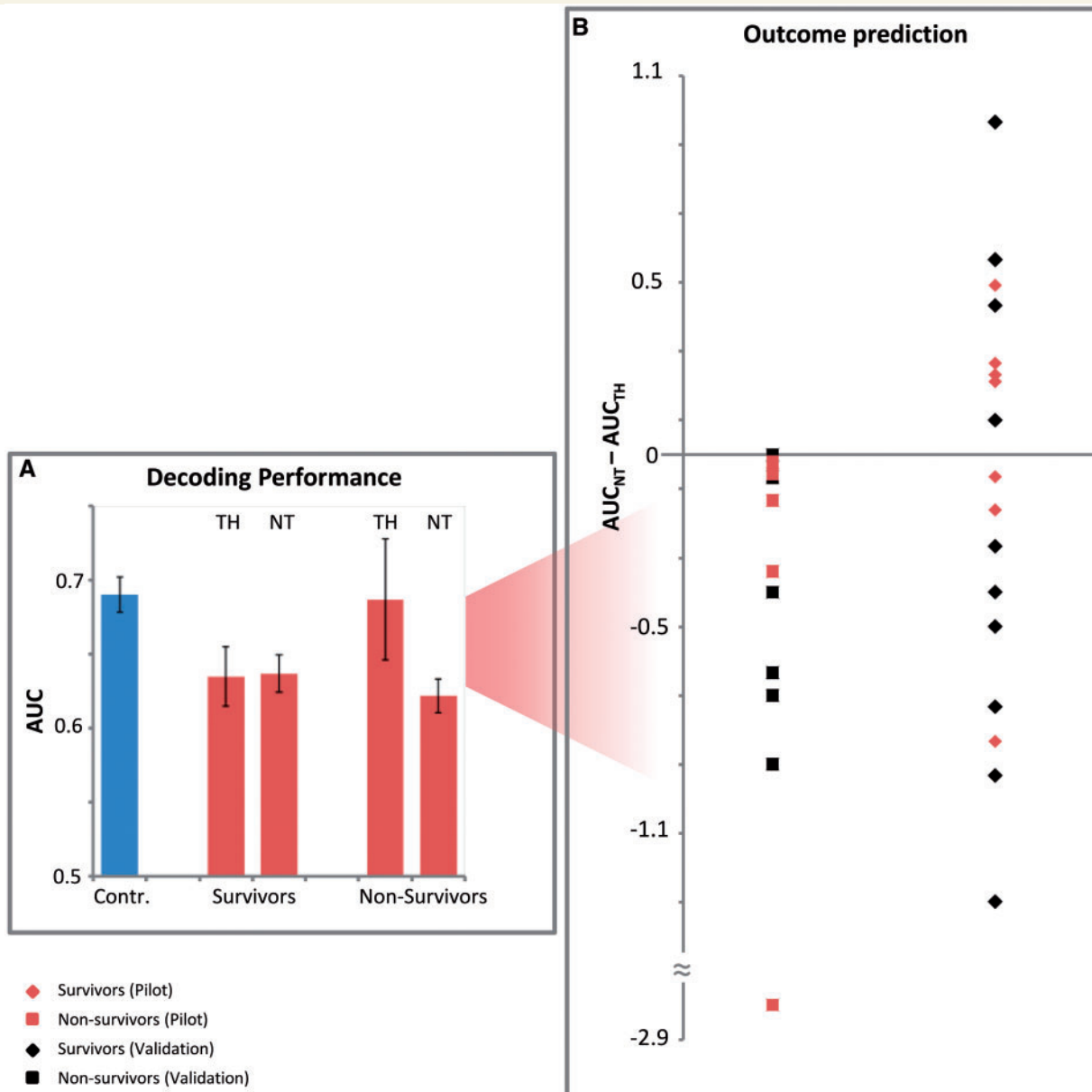
Figure 1 Decoding results and outcome prediction. (**A**) Average decoding performance (SEM indicated) for the five controls (blue) and the pilot group of 12 patients (red). Decoding was measured as the area under the curve (AUC) obtained when discriminating responses to standard versus one type of deviant sounds (in terms of duration, location or pitch). The decoding performance was averaged across the three types of deviants and across patients under therapeutic hypothermia (TH) and under normothermic (NT) conditions. The decoding performance was at similar levels for patients and controls, especially for non-survivors and during therapeutic hypothermia. This decoding performance typically decreases from therapeutic hypothermia to normothermic conditions in non-survivors. (**B**) The difference in decoding performance from therapeutic hypothermia to normothermic conditions, evaluated independently for each patient, was used for predicting the patients' outcome. In the pilot group (red rhombi and squares), an improvement on the average decoding performance from therapeutic hypothermia to normothermic conditions was only observed in survivors (rhombi), resulting in a positive predictive value of 100%. All non-awakening patients showed a drop in their performance (squares). These results were replicated on a validation group of 18 patients (black rhombi and squares), whose data analysis was done blindly to their outcome.

of different neural generators in response to the two sounds categories; a difference in scalp topographies forcibly reflects a difference in the configuration of the underlying generators (Murray *et al*., 2008; Michel and Murray, 2012). Decoding performance was measured as the area under the Receiver Operating Characteristic curve (Green and Swets, 1966), where an area

under the curve value of 1 corresponds to perfect decoding. Importantly, because the analysis was optimized for each patient separately (Tzovara *et al*., 2012a), these decoding performance values cannot be subjected to a group analysis. Therefore, the mean and standard error in Fig. 1 are only shown as a summary of the individual performances.

Average auditory evoked potentials

In addition to the multivariate EEG analysis, we evaluated the presence of mismatch negativity based on average auditory evoked potentials in all our patients during therapeutic hypothermia and normothermic and also in our control subjects. In analogy to previous studies of mismatch negativity in comatose patients (Fischer *et al*., 1999, 2004; Daltrozzo *et al*., 2009) we compared responses to standard versus duration deviant sounds. We considered averages of single trials and first assessed whether an N100 response was present. We performed this analysis in terms of topographic consistency across trials along the 50–200 ms post-stimulus period (topographic consistency test; Koenig and Melie-García, 2010). This test quantifies the degree of consistency of a given topography across trials and at each specific time frame and allows a statistical assessment of the presence of an evoked potential (Koenig and Melie-García, 2010). Among those patients who showed a robust N100 based on the topographic consistency test, we further determined those who showed a statistical difference across trials in frontal electrodes (FP1, FP2 and Fz), in response to standard versus duration deviant sounds (*t*-test, $P < 0.05$). Such an effect cannot physically occur before the sounds start differing, and we therefore considered a temporal window of 100–300 ms, i.e. starting at the end of the standard sound. The presence of statistical differences within this window is a minimal condition for the presence of any differential activity in response to standard and deviant sounds including mismatch negativity (Bekinschtein *et al*., 2009; Boly *et al*., 2011).

Results

The five healthy control subjects showed a robust and accurate auditory discrimination with an average area under the curve value of 0.72 for decoding EEG responses to standard versus duration deviant sounds, 0.71 for standard versus pitch deviants and 0.64 for standard versus location deviants (Fig. 1A). This confirmed that our decoding algorithm reliably discriminated neural responses between standard and deviant sounds.

Auditory discrimination in comatose patients

In the pilot group of 12 patients, the average decoding performance was high for all patients, irrespective of their outcome and for all three types of deviant sounds (Fig. 1A). The best performance was observed during therapeutic hypothermia for non-survivors (Fig. 1A) and was comparable with what was obtained for control subjects on average (Fig. 1A; see Supplementary material for further validation of these results). Moreover, sound discrimination, based on decoding performance, was at similar levels between patients who awoke and those who did not, both under therapeutic hypothermia and normothermic conditions. Importantly, auditory discrimination as measured by the decoding performance was not predictive of final patients' outcome neither under therapeutic hypothermia nor under normothermic conditions (at least at this very early stage of coma).

Prediction of awakening

By contrast, the change in the area under the curve from therapeutic hypothermia to normothermic conditions was predictive of the patients' outcome in the pilot group (Fig. 1B). An increase in the area under the curve from the first (therapeutic hypothermia) to the second (normothermic) recording was only observed in survivors (Fig. 1B), as all patients who did not awake from the coma had unchanged or decreased decoding performance (Fig. 1B). This result was obtained by averaging the area under the curve obtained using the three types of deviants, as this provided the best prediction of awakening. These first results on the pilot group gave 100% positive predictive value, i.e. all patients with an improvement in the decoding performance from therapeutic hypothermia to normothermic conditions awoke from coma and survived at 3 months.

Validation group

We further recorded data from 18 additional consecutive patients (validation group). All data analysis in this validation group was performed blindly to their outcome, ensuring an objective measure of the predictive value of our method. Results based on this validation group confirmed the observations in the pilot group: an improvement in the decoding performance from therapeutic hypothermia to normothermic conditions was only observed in patients waking from coma and surviving at 3 months (Fig. 1B). Overall, in both groups of patients (pilot and validation; see Table 1 for a summary of the patients and recordings) we found that the change of auditory discrimination from therapeutic hypothermia to normothermic conditions accurately predicted the clinical outcome for 21/30 (70% accuracy). Importantly, only those patients who improved their auditory discrimination survived (equivalent to 100% positive predictive value for waking and survival at 3 months). The average decoding performance for survivors, across the three types of deviant sounds, was 0.63 ± 0.01 (mean $\pm$ SEM) during therapeutic hypothermia and 0.63 ± 0.01 during normothermic conditions, while for non-survivors it was 0.67 ± 0.02 during therapeutic hypothermia and 0.63 ± 0.01 during normothermic conditions. Moreover, our results provide evidence of intact neural discrimination between standard and deviant sounds during the early phase of coma, largely independent of patients' outcome (Fig. 2).

Average auditory evoked potentials

Average auditory evoked potentials revealed structured modulations at frontal electrodes for all healthy age-matched control subjects (Fig. 3A for a typical control), but also for some comatose patients (Fig. 3B for a typical comatose patient under normothermic conditions). The topographic consistency test revealed that 23/30 patients during therapeutic hypothermia and 20/30 patients during normothermic conditions had a consistent evoked response during the 50–200 ms temporal window. These results show that including only those patients who show a robust evoked activity at the average auditory evoked potential level would imply a loss of
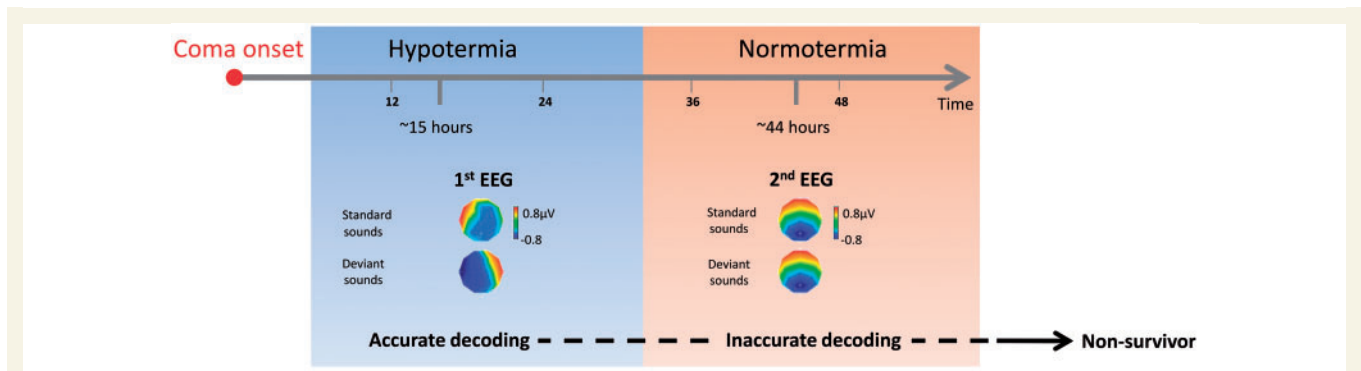
Figure 2 Schematic representation of the procedure and exemplar voltage topographies for a patient who did not awake. The first EEG recording was typically performed at ~15 h after cardiac arrest, during therapeutic hypothermia and the second at ~44 h, after rewarming, under normothermic conditions (Table 1). A worsening in decoding performance was observed in all the patients who did not awake from coma. Typically, based on the first EEG recording during therapeutic hypothermia, we obtained an accurate decoding performance, which resulted from topographic differences in response to standard and deviant sounds (voltage topographies estimated by the single-trial model along 100–300 ms post-stimulus onset; *left* panel). However, during normothermic conditions, the estimated voltage topographies over the same time window did not differ for the two types of sounds (*right* panel) and decoding was not above chance. Such a drop in decoding performance was observed in all patients who do not awake from coma. We conclude that the neural mechanisms responsible for sound discrimination are corrupted after 24 h from coma onset and after the end of therapeutic hypothermia.
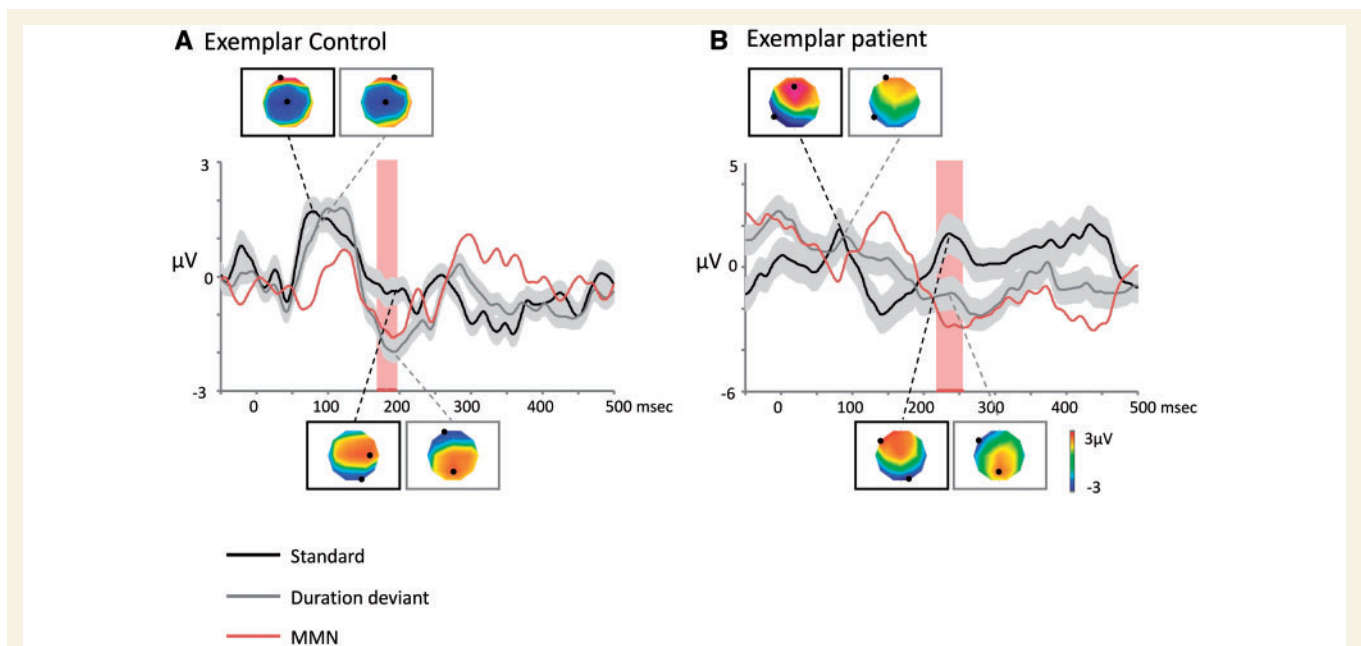

Figure 3 Average auditory evoked potential waveforms (standard error indicated) and scalp voltage topographies for an exemplar healthy control (**A**) and an exemplar patient (**B**). Average auditory evoked potential responses at a group of frontal electrodes (FP1, FP2 and Fz) to standard (black line), duration deviant sounds (grey line) and the difference of the two (red line—mismatch negativity) are plotted as a function of time. The average scalp topographies observed in response to standard and deviant sounds are plotted at the latencies of amplitude peaks (at 78 and 192 ms post-stimulus onset for the control and 81 and 234 ms post-stimulus onset for the patient). The post-stimulus time periods of significant difference (*t*-test; $P < 0.05$) between the two types of responses are indicated by red stripes. These periods were 172–180 ms and 186–195 ms post-stimulus onset for the healthy control (**A**) and 221–263 ms post-stimulus onset for the exemplar patient (**B**). Data in (**B**) were recorded from a patient during normothermic conditions who later died. MMN = mismatch negativity.

almost one-third of the patients, in accordance with what has been reported previously (Fischer *et al.*, 2004).

Among the 23 patients who had a consistent auditory evoked potential to sounds during therapeutic hypothermia, nine (six later awoke, three died) also exhibited a difference between the standard and duration deviant sounds within the 100–300 ms window (see 'Materials and methods' section). Such a difference was also found during normothermic conditions in 3 patients (two later

awoke, one died) out of the 20 who had a consistent evoked response. By contrast, all five control subjects had a topographic consistency within the specified 50–200 ms post-stimulus temporal window, and four of them had a significant difference in response to standard and duration deviant sounds at a group of frontal electrodes within the 100–300 ms post-stimulus onset window.

In summary, during therapeutic hypothermia, 6 out of 14 survivors and three out of nine non-survivors who had an auditory evoked potential also had a statistical difference, while during normothermia 2 out of 14 survivors and one out of six non-survivors who had an evoked response also had a statistical difference. These tests on comatose patients show that mismatch negativity (assessed at average auditory evoked potential at electrodes level, during early coma) was not informative about their final outcome. Of note, when applying baseline correction to average auditory evoked potentials, the number of patients providing evidence of mismatch negativity did not change (10/23 patients had a consistent evoked response during therapeutic hypothermia with baseline correction versus nine without; 2/20 during normothermic had consistent evoked response with baseline correction versus three without). More importantly, baseline correction did not change our ability to predict outcome.

Time between recordings and outcome

The difference in the time between recordings across patients for whom decoding improved versus worsened was nearly significant ($P = 0.08$, Table 1), thus representing a potential confounding factor. Therefore, we considered a subgroup of patients, so that the time between the two recordings did not differ: we selected those for whom this interval was no longer than 25 h. For this supplemental analysis we included 22 patients, 10 with an improvement in decoding (the same we had before) and 12 with a worsening. The time between the two recordings was on average 23 h for both groups (decoding drops versus increases; Mann–Whitney U-test; $P > 0.6$). In this group of 22 patients, we obtained a positive predictive value of 100%, a negative predictive value of 67% and overall accuracy 82%. While positive predictive value remained the same, the negative predictive value and the accuracy were actually higher than those obtained when including all patients (accuracy 70%). Additionally, for all 30 patients, there was no correlation between the difference in the decoding accuracy and the time between the two recordings (Spearman's rho = −0.15, $P = 0.44$). Even when considering the subgroup of patients matched for the time between the two recordings, this correlation was not significant (Spearman's correlation: rho = 0.03, $P = 0.88$).

Discussion

In summary, we showed two novel results. First, we provided evidence of successful sound discrimination during early stages of post-anoxic coma and under therapeutic hypothermia in a large cohort of consecutive patients, independently of their outcome. Even patients who did not awake from coma exhibited differential patterns of EEG activity in response to standard/

deviant sounds (Fig. 1A). Second, we showed that improvement of sound discrimination during the early phase of coma is predictive of awakening and survival at 3 months, with 100% positive predictive value (Fig. 1B and Fig. 2 for an overview and Table 1 for clinical details of the patients).

To the best of our knowledge, this is the first study to report evidence of auditory discrimination in early hours of coma and even in comatose patients who eventually die. Previous studies assessed sound discrimination at variable latencies from coma onset and mostly after several days from the initial insult (Fischer et al., 2004; Naccache et al., 2005) and reported the presence of mismatch negativity responses as a predictor of good outcome (see Daltrozzo et al., 2007 for a meta-analysis). Our results are generally consistent with such previous findings, as they confirm our initial hypothesis that impairment in neural mechanisms for sound discrimination is a process that occurs over time (Figs 1 and 2). Specifically, our results suggest that auditory functions can still be intact during the first day after coma onset, largely independent of the patients' final outcome.

At present, in a clinical routine, prognostication of coma after hypoxic–ischaemic encephalopathy and therapeutic hypothermia relies on a multimodal approach. Specifically, lack of return of brainstem reflexes at 72 h, early myoclonus, and bilateral absence of early cortical somatosensory evoked potentials have robust predictive value for death (Bouwes et al., 2009; Fugate et al., 2010; Rossetti et al., 2010). However, none of these tests is informative of the chances of survival. Our method thus offers a possibility to bridge the prognostic gap as it identifies those patients who will awaken in an automatic and quantitative fashion. Moreover, our method provides early and automatic outcome prediction (within ~48 h after the coma onset) without disregarding any patient from analysis. Importantly, all analyses were done blindly to patients' outcome and were not used at any point for influencing the clinicians' decision for treatment. Clinicians caring for patients were unaware of the results, so that therapeutic attitudes and decisions were not influenced.

For analysing the EEG data, we implemented a single-trial decoding approach that allows assessing the degree of difference in the neural responses to sounds. Our multivariate, data-driven approach for decoding was blind to the patients' outcome and provided similar levels of decoding performance in comatose patients, irrespective of their outcome, and was comparable to that of healthy control subjects. The comparison between decoding results and those obtained at average event-related potential level emphasize the higher sensitivity of multivariate analyses with respect to those obtained at the single electrode level. Differential responses to standard versus duration deviant sounds at a group of frontal electrodes were only observed for three patients who did not awake from coma during therapeutic hypothermia and one during normothermic conditions. However, based on our decoding approach and by taking into account measurements from all electrodes, we found robust evidence of sound discrimination for two extra patients during therapeutic hypothermia (Supplementary material).

The features we used for decoding (i.e. scalp voltage topographies) are indicative of the activity within different neural networks for the processing of standard and deviant sounds.

However, they do not explicitly provide information about what these networks may be or about their difference between controls and patients. Further studies need to be conducted with higher number of electrodes in the EEG set-up which could facilitate the source estimation underlying auditory responses (Michel and Murray, 2012). This aspect would cast light on the possible role of primary auditory and fronto-parietal cortices and the strength of the functional connectivity between them (Boly *et al.*, 2011) as well as on the neural mechanism underlying the differential responses to the three types of deviants (Supplementary material).

Our decoding performance results suggested a better discrimination between standard and duration deviants than the other types of deviants. This evidence is in line with the choice of using duration deviant in other mismatch negativity studies (Fischer *et al.*, 1999, 2004; Daltrozzo, 2009). In addition, the evolution of the decoding performance for the duration deviant was the most informative within the three types of deviants about the patients' outcome (Supplementary Fig. 1 and Supplementary Table 1).

The mismatch negativity paradigm used in our study is similar to those used in previous research on comatose or psychiatric patients (Fischer *et al.*, 2004; Todd *et al.*, 2008) and does not involve inversion of the stimuli (i.e. roving mismatch negativity). We cannot exclude that differences in neural responses to standard versus deviant sounds are also due to low-level features of the stimuli and we therefore interpret our decoding results in terms of sound discrimination in general.

In our study we included patients with similar levels of disorder of consciousness during therapeutic hypothermia and normothermic conditions as measured by the Glasgow Coma Scale. Previous mismatch negativity studies have highlighted a relation between the mechanism underlying the mismatch negativity (and its strength) and the consciousness level. However, our study suggests that even patients with a similar level of unconsciousness can exhibit different degrees of auditory discrimination (higher during therapeutic hypothermia than during normothermic conditions) and therefore are likely to have different underlying mechanisms. As a potent inhibitor of brain excitotoxicity (Dietrich and Bramlett, 2010), hypothermia has many neuroprotective properties (Yenari and Han, 2012), therefore, we speculate that therapeutic hypothermia may reduce the physiological background noise, thereby allowing a more reliable measure of the evoked response to incoming stimuli. Whatever the precise cause of this phenomenon, we find these data intriguing and worth further study to better analyse ongoing background activity and spatio-temporal mechanisms underlying auditory discrimination in hypothermic conditions. In summary, our study shows that early assessment of auditory functions based on EEG multivariate analyses promises to provide a highly informative test of the chance of survival of comatose patients and to largely revise our understanding of intact cerebral functions in patients with disorder of consciousness.

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Supplementary material

Supplementary material is available at *Brain* online.

References

American Heart AssociationGuidelines for cardiopulmonary resuscitation and emergency cardiovascular care. Circulation 2005; 112: IV1–203.

Bekinschtein TA, Dehaene S, Rohaut B, Tadel F, Cohen L, Naccache L. Neural signature of the conscious processing of auditory regularities. Proc Natl Acad Sci USA 2009; 106: 1672–7.

Bernasconi F, De Lucia M, Tzovara A, Manuel A, Murray MM, Spierer L. Noise in brain activity engenders perception and influences discrimination sensitivity. J Neurosci 2011; 31: 17971–81.

Boly M, Garrido MI, Gosseries O, Bruno MA, Boveroux P, Schnakers C, et al. Preserved feedforward but impaired top-down processes in the vegetative state. Science 2011; 332: 858–62.

Bouwes A, Binnekade JM, Zandstra DF, Koelman JH, van Schaik IN, Hijdra A, et al. Somatosensory evoked potentials during mild hypothermia after cardiopulmonary resuscitation. Neurology 2009; 73: 1457–61.

Brunet D, Murray MM, Michel CM. Spatiotemporal analysis of multichannel EEG: CARTOOL. Comput Intell Neurosci 2011; 2011: 813–70.

Cruse D, Chennu S, Chatelle C, Bekinschtein TA, Fernández-Espejo D, Pickard JD. Bedside detection of awareness in the vegetative state: a cohort study. Lancet 2011; 378: 2088–94.

Daltrozzo J, Wioland N, Mutschler V, Kotchoubey B. Predicting coma and other low responsive patients outcome using event-related brain potentials: a meta-analysis. Clin Neurophysiol 2007; 118: 606–14.

Daltrozzo J, Wioland N, Mutschler V, Lutun P, Calon B, Meyer A, et al. Cortical information processing in coma. Cogn Behav Neurol 2009; 22: 53–62.

De Lucia M, Michel CM, Clarke S, Murray MM. Single subject EEG analysis based on topographic information Proceedings of the IEEE/EMBS Region 8 International Conference on Information Technology Applications in Biomedicine, ITAB, art. no. 4407353, 2007.

De Lucia M, Michel CM, Murray MM. Comparing ICA-based and single-trial topographic ERP analyses. Brain Topogr 2010; 23: 119–27.

De Lucia M, Tzovara A, Bernasconi F, Spierer L, Murray MM. Perceptual decision making of environmental sounds. Neuroimage 2012; 60: 1704–15.

Dietrich WD, Bramlett HM. The evidence for hypothermia as a neuroprotectant in traumatic brain injury. Neurotherapeutics 2010; 7: 43–50.

Faugeras F, Rohaut B, Weiss N, Bekinschtein TA, Galanaud D, Puybasset L, et al. Probing consciousness with event-related potentials in the vegetative state. Neurology 2011; 77: 264–8.

Fischer C, Morlet D, Bouchet P, Luaute J, Jourdan C, Salord F. Mismatch negativity and late auditory evoked potentials in comatose patients. Clin Neurophysiol 1999; 110: 1601–10.

Fischer C, Luauté J, Adeleine P, Morlet D. Predictive value of sensory and cognitive evoked potentials for awakening from coma. Neurology 2004; 63: 669–73.

Fugate JE, Wijdicks EF, Mandrekar J. Predictors of neurologic outcome in hypothermia after cardiac arrest. Ann Neurol 2010; 68: 907–14.

Garrido MI, Kilner JM, Stephan KE, Friston KJ. The mismatch negativity: a review of underlying mechanisms. Clin Neurophysiol 2009; 120: 453–63.

Green DM, Swets JM. Signal detection theory and psychophysics. New York: John Wiley and Sons Inc; 1966.

Holzer M. Targeted temperature management for comatose survivors of cardiac arrest. N Engl J Med 2010; 363: 1256–64.

Kane NM, Curry SH, Butler SR, Cummins BH. Electrophysiological indicator of awakening from coma. Lancet 1993; 341: 688.

Koenig T, Melie-García L. A method to determine the presence of averaged event-related fields using randomization tests. Brain Topogr 2010; 23: 233–42.

Kotchoubey B, Lang S, Mezger G, Schmalohr D, Schneck M, Semmler A, et al. Information processing in severe disorders of consciousness: vegetative state and minimally conscious state. Clin Neuropysiol 2005; 116: 2441–53.

Michel CM, Koenig T, Brandeis D, Gianotti LRR, Wackermann J, editors. Electrical neuroimaging. Cambridge: Cambridge University Press, 2009.

Michel CM, Murray MM. Towards the utilization of EEG as a brain imaging tool. Neuroimage 2012; 61: 371–85.

Murray MM, Brunet D, Michel CM. Topographic ERP analyses: a step-by-step tutorial review. Brain Topogr 2008; 20: 249–64.

Näätänen R, Gaillard AW, Mäntysalo S. Early selective-attention effect on evoked potential reinterpreted. Acta Psychol 1978; 42: 313–29.

Naccache L, Puybasset L, Gaillard R, Serve E, Willera JC. Auditory mismatch negativity is a good predictor of awakening in comatose patients: a fast and reliable procedure. Clin Neurophysiol 2005; 116: 988–9.

Neumann N, Kotchoubey B. Assessment of cognitive functions in severely paralysed and severely brain-damaged patients: neuropsychological and electrophysiological methods. Brain Res Protocols 2004; 14: 25–36.

Oddo M, Schaller MD, Feihl F, Ribordy V, Liaudet L. From evidence to clinical practice: effective implementation of therapeutic hypothermia to improve patient outcome after cardiac arrest. Crit Care Med 2006; 34: 1865–73.

Perrin F, Pernier J, Bertrand O, Giard MH, Echallier JF. Mapping of scalp potentials by surface spline interpolation. Electroencephalogr Clin Neurophysiol 1987; 66: 75–81.

Rossetti AO, Oddo M, Logroscino G, Kaplan PW. Prognostication after cardiac arrest and hypothermia: a prospective study. Ann Neurol 2010; 67: 301–7.

Todd J, Michie PT, Schall U, Karayanidis F, Yabe H, Näätänen R. Deviant matters: duration, frequency, and intensity deviants reveal different patterns of mismatch negativity reduction in early and late schizophrenia. Biol Psychiatry 2008; 63: 58–64.

Tzovara A, Murray MM, Plomp G, Herzog MH, Michel CM, De Lucia M. Decoding stimulus-related information from single-trial EEG responses based on voltage topographies. Pattern Recogn 2012a; 45: 2109–22.

Tzovara A, Murray MM, Bourdaud N, Chavarriaga R, Del R, Millán J, De Lucia M. The timing of exploratory decision-making revealed by single-trial topographic EEG analyses. Neuroimage 2012b; 60: 1959–69.

Tzovara A, Murray MM, Michel C, De Lucia M. A tutorial review of electrical neuroimaging from group-average to single-trial event-related potentials. Dev Neuropsychol 2012c; 37: 518–44.

Yenari MA, Han HS. Neuroprotective mechanisms of hypothermia in brain ischaemia. Nat Rev Neurosci 2012; 13: 267–78.

Wijnen VJ, van Boxtel GJ, Eilander HJ, de Gelder B. Mismatch negativity predicts recovery from the vegetative state. Clin Neurophysiol 2007; 118: 597–605.