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Predicting the outcome of post-anoxic comatose patients based on single-trial EEG analysis

Simonin Alexandre

UNIVERSITE DE LAUSANNE – FACULTE DE BIOLOGIE ET DE MEDECINE

Département des Neurosciences Cliniques

Centre Hospitalier Universitaire Vaudois

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THESE

préparée sous la direction de la Docteure Marzia de Lucia
avec la co-direction du Professeur Marc Levivier

et présentée à la Faculté de biologie et de médecine de
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par

Alexandre Simonin

Médecin diplômé de la Confédération Suisse
Originaire de Genève (GE) et de Les Bois (JU)

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Predicting the outcome of post-anoxic comatose patients based on single-trial EEG analysis

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Madame la Professeure Stephanie Clarke
Directrice de l'Ecole doctorale

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1. Résumé

Les maladies cardio-vasculaires représentent la première cause de mortalité en Suisse. Après un arrêt cardio-respiratoire, une minorité des patients survit sans ou avec peu de séquelles fonctionnelles. L'évaluation du pronostic se fait classiquement selon des critères établis par l'Académie Américaine de Neurologie (AAN) en 2006, soit précédant l'introduction de l'hypothermie thérapeutique. Depuis, ces critères semblent insuffisants, et de nouveaux examens para-cliniques sont nécessaires afin d'identifier les patients ayant un pronostic favorable.

La détection d'irrégularités auditives, et plus particulièrement l'évolution de cette détection sur plusieurs jours, pourrait être un indicateur du pronostic de patients comateux suite à une anoxie cérébrale. En effet, lors d'une violation de la régularité établie par des séries de sons identiques, deux signaux sont détectables à l'électro-encéphalographie (EEG). Le premier, dénommé «Mismatch negativity» (MMN), peut être enregistré après une violation *locale* d'une régularité établie au niveau de chaque son. Il reflète un processus inconscient et ne demandant pas de ressources attentionnelles. Le deuxième, dénommé « complexe P300 » survient par contre après une violation *globale* d'une régularité établie au niveau de groupes de sons. La littérature actuelle indique que ce deuxième phénomène requerrait la présence de capacités attentionnelles.

Dans notre étude, nous avons testé l'existence de cette détection d'irrégularités auditives globales chez des patients dans une phase précoce de coma post-anoxique, sous hypothermie thérapeutique. Nous avons enregistré la réponse électro-encéphalographique lors de violations de régularités auditives globales, à l'aide d'un protocole expérimental qui intégrait en plus un paradigme de MMN classique, afin de tester la détection d'irrégularités auditives locales également. Notre analyse finale inclut 24 patients comateux ayant subi un arrêt cardio-respiratoire, et bénéficié du protocole hypothermie du Centre Hospitalier Universitaire Vaudois (CHUV) à Lausanne. Après une analyse multivariée des réponses électro-encéphalographiques de chaque tracé individuellement (« single-trial »), nous avons trouvé que 8 patients sur 24 pouvaient discriminer une irrégularité globale, alors qu'étant définis comateux selon l'échelle de Glasgow (GCS). De plus, l'amélioration de la détection d' irrégularités auditives entre deux EEG consécutifs (en hypo- puis normothermie), était un facteur de bon pronostic. Notre test pourrait ainsi être un complément para-clinique dans l'évaluation du pronostic de patients en coma post-anoxique.

2. Introduction

In Switzerland, cardiovascular disease accounts for around 30% of all deaths per year [1]. With resuscitation after cardiac arrest, approximately half of patients recover spontaneous circulation [2]. Before therapeutic hypothermia (TH), only 20% of patients survived. [3] Among patients experiencing a cardiac arrest, less than 10 % will regain consciousness [4].

Therapeutic hypothermia improved outcome after adult cardiac arrest over the past decade [5,6]. However, outcome prediction of comatose patients following cardiac arrest relies on clinical and electroencephalographic (EEG) prognostic markers, with guidelines established by the American Academy of Neurology (AAN) in 2006, that were developed before therapeutic hypothermia was introduced. Recent studies [2,5,6,7] criticize some of these guidelines, and describe patients treated with hypothermia regaining consciousness, in spite of poor outcome prediction based on AAN guidelines [7], especially absent or extension motor response after 3 days, serum neuron-specific enolase (NSE) values greater than 33 μg/L within 3 days, and electrographic status epilepticus. Moreover, all prognosticators described in those guidelines are intended to predict poor outcome. Therefore, clinicians need adapted outcome prediction tools, especially neurophysiological tests, targeting a high predictive value for good outcome.

An extensive literature on the so-called mismatch negativity (MMN) paradigms has provided evidence of the relation between auditory responses as measured by EEG and patients' chance of surviving (e.g.[10][14]). In the auditory domain, two different neural responses are detected on EEG when a violation of an auditory regularity occurs, either at the level of single sounds (local regularity) or groups of sounds (global regularity)[15]. First, violations of local regularities, or MMN (Figure 1) (i.e. when there is a deviant within a series of five sounds for example), can be measured independently of subjects' awareness of the auditory regularity [15,33].

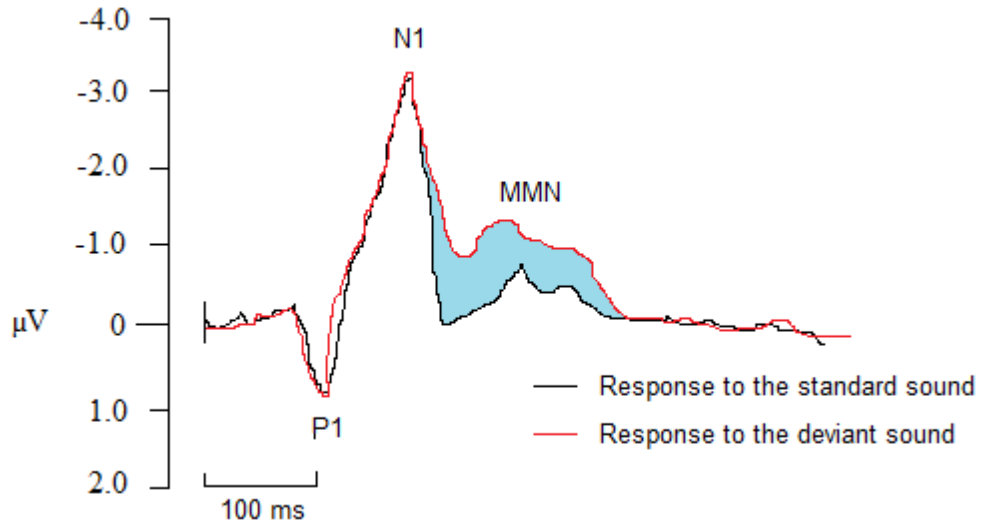

Figure 1 : Average auditory evoked potentials (ERPs) as response to standard or deviant sounds in a mismatch negativity paradigm (simulated data)

The MMN is thought to be generated by an automatic mechanism, mainly in the auditory cortex, depending on the physical features of a sound, and it can be measured even in the absence of attention [11]. Therefore, it can be used even with patients unable to interact, for example comatose, vegetative state (VS), or minimally conscious state (MCS) patients [12]. By contrast, detection of global regularities violations (i.e. when there is a deviant across sound series) elicits another later response, labeled P300 (P3a and P3b) complex [13]. While the MMN reflects a nonconscious response not requiring attention, the P300 complex has been shown to depend on subjects' awareness of the regularity [15], and irrespective of the physical differences of the stimuli [14]. In a recent study [18], this later neural response has been reported to be a specific signature of conscious processing. Moreover, VS patients showing this response presented clinical signs of consciousness within 3 to 4 days following evoked-related potential (ERP) recording [18]. Finally, a meta-analysis [14] and a more recent study [31] showed that MMN and P300 are predictive of awakening among comatose patients.

However, most of previous studies recording those ERP components were performed among an heterogeneous comatose population, including hemorrhagic or ischemic stroke syndromes, traumatic brain injury, respiratory or cardiac failure, complications of vascular or tumoral neurosurgery, and encephalitis [10]. Moreover, they were performed in normothermic conditions, before therapeutic hypothermia became the standard of care for those patients [5].

In the present study, we focused on post-anoxic comatose patients following cardiac arrest, treated with mild TH after resuscitation, according to a standardized procedure [5]. We implemented an auditory oddball paradigm inspired by Bekinschtein et al. [15] in order to elicit MMN and P300 components within the same trials. This auditory oddball paradigm uses 2 levels of auditory regularity ; one is induced by the repetition of identical sounds (local regularity), the other by repetition of groups of sounds (global regularity). Interruption of the local regularity (local deviant) was correlated in Bekinschtein's studies with two electrical successive events: first a mismatch negativity, centered at the vertex, appearing about 130 ms after presentation of the local deviant sound, followed by occipito-temporal negativities simultaneous to a central positivity going from 200 to 300 ms. By contrast, interruption of the global regularity correlated with a frontal negativity simultaneous to a central positivity, occuring 260 ms after the presentation of the global deviant sound, and lasting until 700 ms. They showed that 100% of subjects actively counting the global deviants presented a global effect. By contrast, none of the 4 VS patients had a global effect. Therefore, the authors concluded that detection of global auditory irregularities might be more than a simple indicator of vigilance, reflecting subjective conscious contents [15,32-34]. On the other hand, the detection of local auditory irregularities could reflect a preserved nonconscious integration of auditory environment, which does not imply conscious perception.

However, little is known about the validity of these statements during therapeutic hypothermia. Some of our colleagues performed a study [17], hypothesizing that auditory functions might still be preserved during early coma and under therapeutic hypothermia and that an absence of local effect (MMN) could result from the progressive degeneration of auditory processing over time. Their results showed that auditory processing was intact in comatose patients under therapeutic hypothermia, even in those who did not survive. However, improvement of auditory processing over time was informative of the chance of surviving: a progression of sound discrimination between therapeutic hypothermia and normothermia recordings was only observed in survivors.

Here, we made the hypothesis that a violation detection in a high level auditory oddball paradigm presenting global auditory irregularities, can also be intact during the early days of coma, and that its progression over time can be predictive of patients' outcome. We implemented a similar protocol [17], adapted to include only duration deviants, as it has been shown to be the most sensitive physical sound feature for MMN recording [8,19]. Moreover, we used a paradigm with four different trials, using the same sounds but in different orders, (see Material and Methods) in order to control sounds for

the local MMN and physical features differences, while presenting global auditory irregularities. Finally, we used a multivariate EEG decoding approach developed by some of our collaborators [16] measuring the voltage distribution over the whole electrode montage, allowing to analyze neural responses for each patient separately, without a priori hypothesis concerning the magnitude and latency of auditory evoked potentials (AEP) responses. This kind of analysis is particularly suitable for clinical studies, where patients do not exhibit components at the average AEP level, and where the inter-subject variability does not allow carrying out group analysis [17]. An improvement of decoding accuracy between the two recordings could be informative of intact high-level auditory processing, predicting a favourable outcome among post-anoxic comatose patients treated with therapeutic hypothermia.

3. Methods

Patients and controls

Our study included 30 post-anoxic comatose patients admitted from November 2011 to August 2012 to the Department of Critical Care Medicine, Centre Hospitalier Universitaire Vaudois (CHUV), Lausanne, Switzerland. Of these, 1 patient died before the second recording, and 5 patients presented too many EEG artifacts (mainly epileptic patterns). Finally, 24 patients (6 women, 18 men; mean age 64.88 ± 2.44 years old, average ± s.e.m., range 45-87) were included in the final analysis. Time interval from cardiac arrest to Return of Spontaneous Circulation (ROSC) was 20.71 ± 11.04 minutes, average ± s.e.m., range 5-50.

After resuscitation from cardiac arrest, all were treated with mild TH to 33-34°C for 24 hours using intravenous ice-cold fluids, ice-packs, and a surface cooling device (Arctic Sun System, Medivance, Louisville, CO, USA) for the maintenance of TH. Fentanyl (1.5 µg/kg/h) and midazolam (0.1 mg/kg/h) were administered for sedation, and vecuronium (0.1 mg/kg boluses) to avoid shivering. All patients were unconscious during these first 48 hours, with 3 to 4 points on the Glasgow Coma Scale (GCS) assessed every two to three hours. Five of the 24 patients had status epilepticus or myoclonus and received intravenous antiepileptic drugs, which were stopped if there was no improvement after 72 hours. Withdrawal of intensive treatment was discussed if at least two of the following

criteria were present (assessed in normothermia, off sedation, and 48-72 hours at least after cardiac arrest): absence of brainstem reflexes, myoclonus, absent bilateral somatosensory evoked potentials (SSEPs) and non-reactive EEG background to stimulation [2]. The present results were not used for the decision. Survival was evaluated at three months, and outcome was assessed using the Cerebral Performance Categories Scale (CPC) score [24] (See Results).

The final analysis included 24 patients without *a priori* selection based on the presence of an ERP. In addition, we recorded 14 control subjects: 7 in passive conditions (simply listening to the sounds) and 7 in active conditions (counting the deviants). The same experiment and analysis were carried out on controls in order to determine the accuracy of sound discrimination in healthy subjects. None of the control subjects had hearing problems or history of psychiatric or neurological illnesses. For comatose patients, we could not ensure that they had no hearing or neurological problems.

Stimuli

We performed an auditory MMN paradigm using one standard and one type of duration deviant sound. Series of 5 sounds were presented via earphones to the patients or control subjects. Identical (= A) or deviant (= B) sounds consisted of pure 1000 Hz sinusoidal tones, with a duration of 100 (A) or 150ms (B) and 0 ms inter-aural difference. Inter-Stimulus Interval (ISI) of 490ms was constant within series of sounds. Time interval between groups of 5 sounds varied, ranging from 1350 to 1650ms.

There were 4 different group of sounds, either composed by 5 identical sounds (AAAAA or BBBBB) or composed by 4 identical sounds and 1 locally deviant (AAAAB or BBBBA). We presented 4 blocks, each composed by 120 series of 5 sounds (100 out of 120 were standard). Blocks were presented in a way to induce not only a local regularity (i.e. between the fourth and fifth sound of each series), but also a global regularity (i.e. across series of sounds). The first block included AAAAA series for standard, and AAAAB for deviant. The second block inversed series (i.e. AAAAB for standard, AAAAA for deviant). The third and fourth block had the same structure as the first and second, but A and B sounds were reversed, in order to adjust for physical sound properties (Figure 2). In total, we presented 120 sequences in each block (100 standards). As such, a block of trials consisted of 600 sounds and lasted approximately 7 minutes. The whole protocol and

continous EEG lasted approximately 30 minutes for each patient and control, and was performed in hypothermia and normothermia for each patient.

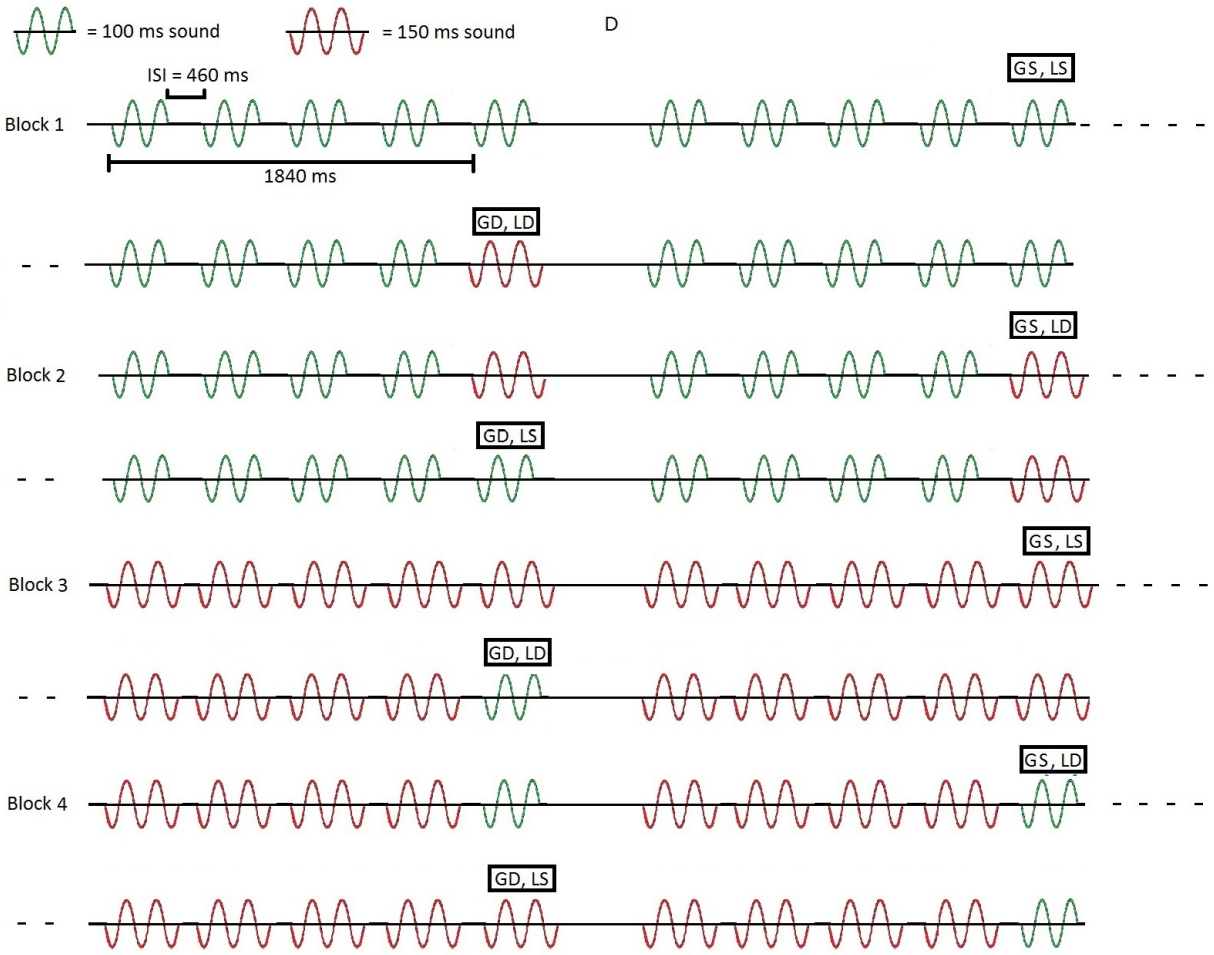

Figure 2 : Experiment paradigm design. GS = Global standard; GD = Global Deviant; LS = Local standard; LD = Local deviant

EEG acquisition and preprocessing

We recorded a continuous EEG (Viasys Neurocare) during hypothermia and another one during normothermia. We used a 19 electrodes montage arranged according to the international system (Figure 3), as used for clinical purposes in the patients. We used a sampling rate of 1024 Hz with the reference set at Fpz. The impedances of all electrodes were maintained below 10 kΩ. All the EEGs were recorded at bedside, in the

clinical environment without disturbing the clinical routine.

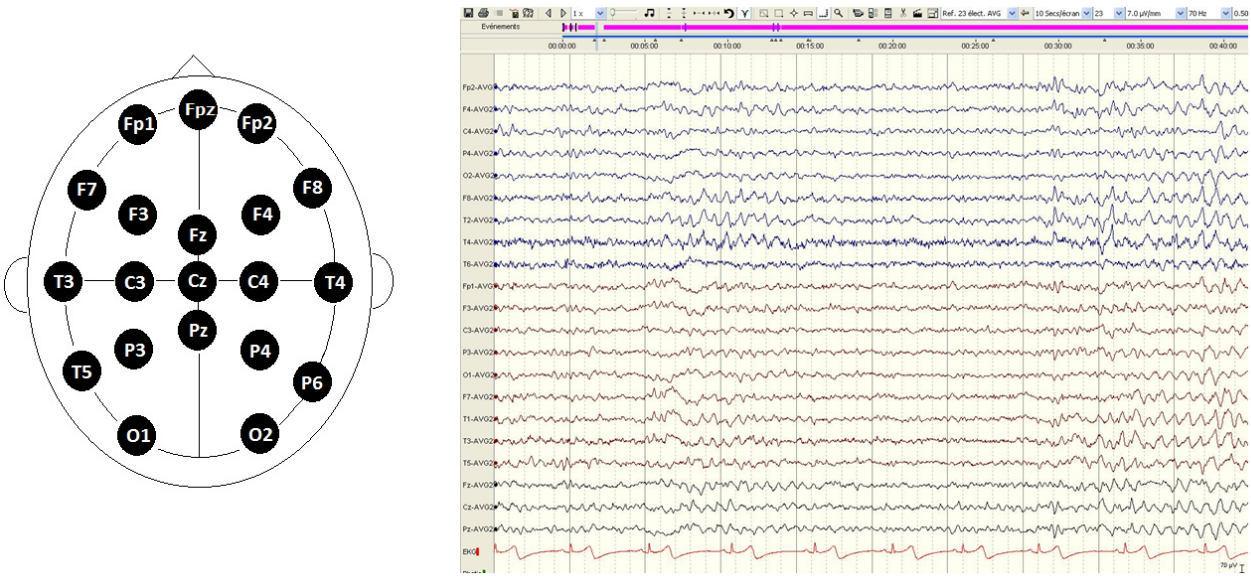

Figure 3 : *Left panel :* EEG international 10-20 electrodes montage. *Right panel* : EEG recording.

To achieve greater consistency, EEG recordings were performed with the same procedure and equipment on control subjects. Healthy subjects, while lying on an inclined chair in a quiet hospital room, were asked to keep their eyes closed and to listen to the sounds. The subjects completing the active task were asked to count the deviants mentally, and reported their counting after each block of experiment. Peri-stimulus EEG signals were extracted, from 100 ms before stimulus onset to 500 ms after sound onset. The data were filtered with 0.1-40 band-pass and 50 Hz notch. An artefact rejection criterion of ± 100 µV was applied offline, and no baseline correction was performed.

Multivariate decoding

Differences in neural responses between standard and deviant sounds were quantified using a multivariate EEG processing [16]. One of the advantages of this statistical analysis is that it is not influenced by preliminary hypotheses concerning the specific scalp location at which a stimulus-related signal is expected. Therefore, it provides a tool to measure response differences at a single-patient level, without *a priori* criteria. This technique uses a Mixture of Gaussians (Figure 3) as a model for the voltage

topographies of the ERPs at a single-trial level.

Another advantage of this analysis is that discrimination accuracy obtained in this framework reflects the activity of different neural systems responding to the standard or deviant sounds. Different scalp topographies results from a different configuration of the neural systems being activated [25]. As such, sound discrimination performance reflects different brain responses to the two categories of sounds in a single-trial setting.

The procedure is divided into a cross-validation (CV) and a validation (V) phase. During the CV, a model allowing an optimal discrimination of EEG responses between standard and deviant sounds is trained and tested. During model training, template maps, representing topographies of the scalp, are extracted for each condition (deviant and standard) at a local (inside each serie of 5 sounds) and at a global level (between series), respectively (Figure 4). This is done on one part of the CV dataset (training set). Using the model, trials of the remaining part of the CV dataset are then assigned to one of the two sets of template maps generated. In other words, using the representative topography, we decode, at a global and local level, if there was a standard or deviant sound presented to the patient on every trial.

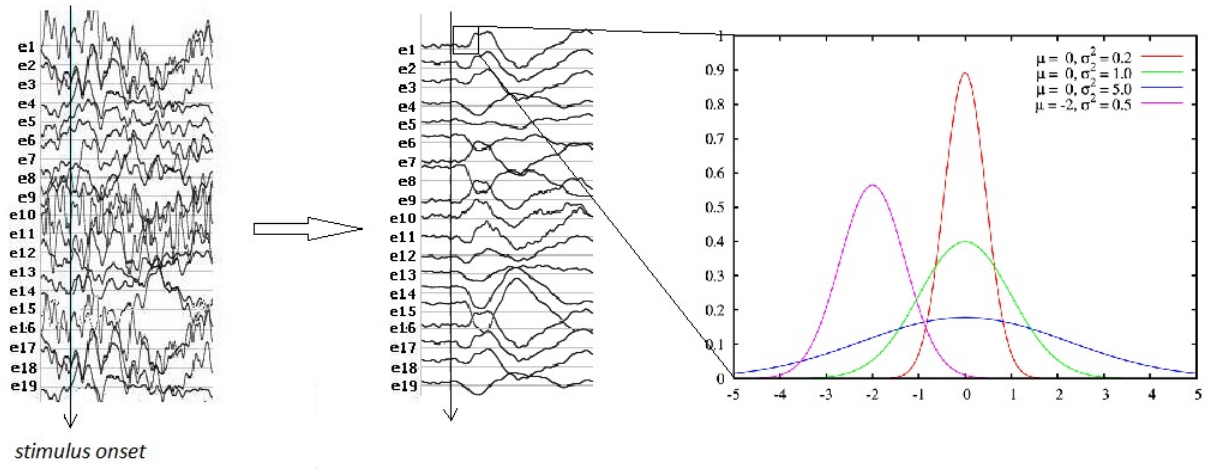

Figure 4: Modelling the voltage topographies using a Mixture of Gaussians, at a single-trial level. *Left panel :* A single trial in response to a sound as recorded at 19 EEG electrodes. *Central panel :* Average auditory evoked potential for the same category of sound in a block of trials. *Right panel:* modelling of an auditory evoked potential as a Mixture of three Gaussians.

The decoding performance is then estimated based on the Receiving Operating Characteristic (ROC) curve when classifying EEG responses to standard or deviant sound.

This procedure is repeated with different sets of template maps in order to find an optimal model, namely the one maximizing the area under the ROC curve across test datasets. In the V phase, the model selected during the CV phase is used to classify trials of the remaining dataset (V dataset) as standard or deviant, at a local and at a global level, respectively. Of course, the V dataset was never used for selecting the models' parameters. Then, for establishing the significance of the Area Under Curve (AUC) values obtained with the V datasets, we ran a permutation test. It consists in randomly permuting the trials of the CV datasets between the two experimental conditions and evaluating two new gaussian mixture models (GMM) for each permutation. The permutation is done 100 times. These two sets of models are used for classifying the validation dataset. The decoding accuracy obtained with the permuted trials is compared to the decoding accuracy with the true trials partition in order to verify that the decoding accuracy obtained in V is better than expected by chance (i.e. better than that of the 100 permuted CV datasets). Classification accuracy was compared to the distribution of those 100 random ones with a Wilcoxon signed-rank test ($p<0.001$).

The number of trials included in the CV was always 60 in all comparisons between EEG responses. The rest of the trials were included in the V dataset. Because of this constraint of having enough artifact-free trials in the CV dataset, we had to completely exclude data from 5 patients due to the presence of artifacts. The analysis was done in separate ways for each of the remaining 24 patients and for each of the two recordings (hypothermic and normothermic conditions). Within these 24 patients, we evaluated first a pilot group including 12 individuals in order to test the validity of the prediction.

4. Results

For every patient and controls, we compared responses to deviant versus standard sounds, at a local and global level separately. As presented in the introduction, our main interest was to evaluate the global MMN effect which has never been explored systematically during early coma and under therapeutic hypothermia. As such, although our paradigm allowed measuring the local MMN as well, we will only describe the global MMN effect.

Healthy controls

We recorded 14 control subjects: a first group of 7 healthy controls in passive conditions (simply listening to the sounds while a continous EEG was recorded) and a second group of 7 healthy controls in active conditions (counting the deviants during EEG). We quantified the sound discrimination at a global level, for each healthy control independently. To do this, we compared responses to the last sound of each group of 5 sounds, in order to adjust for possible duration bias or local effect (MMN) in each group of sounds. In the first passive group, only one subject (out of 7) presented a significant global level discrimination, with a decoding performance of 0.59. In the second group (active counting) by contrast, three controls (out of 7) showed accurate sound discrimination with a decoding performance of 0.68. Interestingly, these three subjects presented the best accuracy in the counting task, with an average of 89 ± 7 % of correct responses. Globally, the second group (active counting) presented an average correct count of 81 ± 5 % (range 61 to 98). Based on the multivariate analysis, passive subjects showed a significant differential activity for global discrimination during the 108-156ms post-stimulus onset period. By contrast, all the subjects of the second group with a significant sound discrimination showed differential responses at 400 ms post-stimulus onset.

Auditory discrimination in comatose patients

Eight out of 24 patients showed a significant discrimination of global standard versus global deviant sounds (Wilcoxon test; $p<0.001$). Among them, three were in TH, four in NT and one in both recordings. To examine whether auditory discrimination was indicative of the outcome, we evaluated first a pilot group, consisting of the 12 first patients, whether their results were significant or not. In this group, the average decoding performance was 0.59±0.01 during TH and 0.60±0.02 during NT for patients surviving. For non-survivors, the mean AUC was 0.64±0.02 during TH and 0.59±0.02 during NT (Figure 6). As such, those with poor outcome decreased their decoding performance between the two recordings. In the pilot group, all patients with a decoding performance improvement between therapeutic hypothermia and normothermia awoke from coma and survived at 3 months, i.e. positive predictive value (PPV)= 100%.

When analyzing the 12 remaining patients, all except two patients with poor outcome showed a significant decrease of AUC between hypothermia and normothermia. The decoding performance for survivors was 0.61±0.01 in TH and 0.59±0.02 in NT. For non-

survivors, the average AUC was 0.60± 0.01 during TH and 0.58±0.02 during NT. This means that all non-survivors but two decreased their decoding performance between the two recordings, indicating a positive predictive value of 78% (Figure 6).

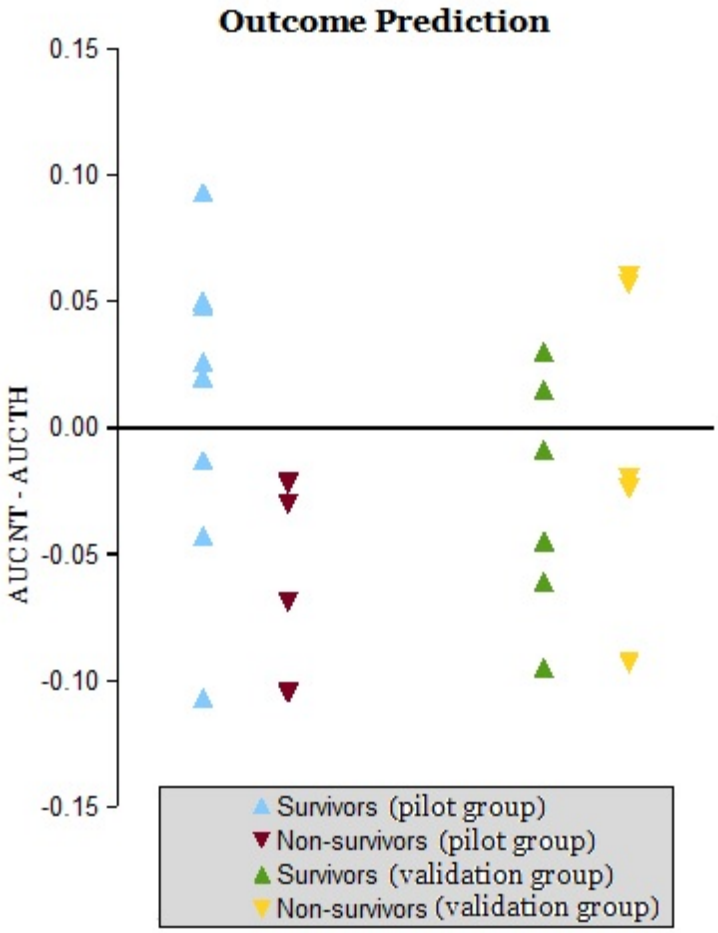

Figure 6 : Decoding performance of each patient, in hypothermia or normothermia, regarding outcome. Plots over the horizontal line represent patients who improved their decoding performance in normothermia (AUC NT > AUC TH). Patients improving their auditory discrimination between recordings survived, except two (yellow triangles).

	Decoding improves	Decoding drops	p-value	t-value
Patients alive at 3 months, %	78	50		
Patients deceased at 3 months, %	22	50		
Age, years	60±22	64±14	0.37	0.68
Time to ROSC (return of spontaneous circulation), min	19±12	22±11	0.39	0.5
Time to first EEG, h	13±4	18±4	0.23	0.88
Time between recordings, h	26±6	22±9	0.79	0.6

Table 1 : Patients description. Results of the comparison of patients' characteristics between the group of patients who improved in their decoding performance vs those who did not (c.f. Figure 6). We detail three-months survival, mean age, duration of cardiac arrest (ROSC), timing of the first EEG recording, time between recordings. P-value and t-value (unpaired t-test) are shown. Values are mean ± SEM.

	Significant results	Non significant results	p-value	t-value
Patients alive at 3 months, %	67	65		
Patients deceased at 3 months, %	43	35		
Age, years	64±9	65±13	0.47	0.75
Time to ROSC (return of spontaneous circulation), min	23±5	20±12	0.77	0.55
Time to first EEG, h	14±5	13±4	0.63	0.74
Time between recordings, h	28±10	27±7	0.64	0.72

Table 2 : Patients description. Results of the comparison of patients' characteristics between those showing statistically significant data vs those who do not. We detail three-months survival, mean age, duration of cardiac arrest (ROSC), timing of the first EEG recording, time between recordings. P-value and t-value (unpaired t-test) are shown. Values are mean ± SEM.

Including only the CV, patients with good outcome showed a mean AUC of 0.60 ± 0.01 in TH and 0.61 ± 0.01 in NT. Patients with poor outcome showed a mean AUC of 0.59 ± 0.01 in TH and 0.59 ± 0.01 in NT (Figure 7). Importantly, sound discrimination performance was similar between patients with a favorable outcome or not, both under therapeutic hypothermia and normothermia. Decoding accuracy was not predictive of patients' outcome in neither conditions (hypo- or normothermia).

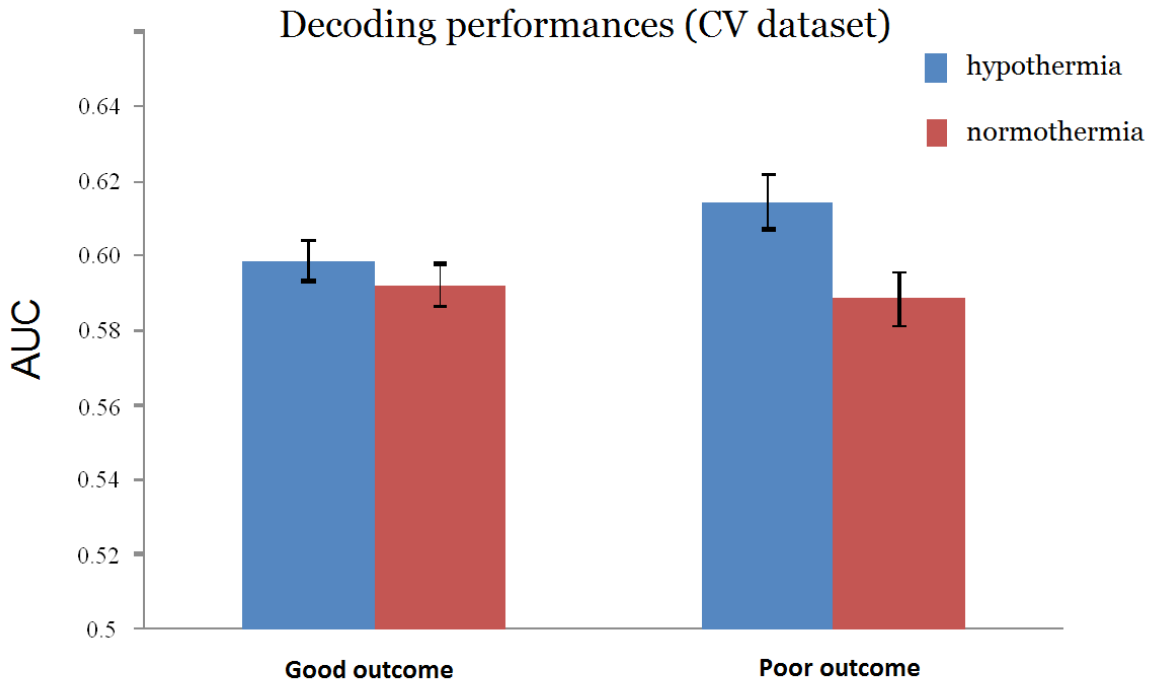

Figure 7 : Decoding performances for the CV dataset

We then compared predictive performances of our test with usual clinical and electrophysiological data routinely used to predict bad outcome : absence of brainstem reflexes, absence of motor response after 3 days, early myoclonus, absence of somatosensory evoked potential (SSEP), Neuron-specific enolase (NSE) > 33 µg/l, unreactive EEG background to stimulation in normothermia. Our test did not provide a strong predictive value for poor outcome, i.e. negative predictive value (NPV) = 53%, meaning that in case of worsening of decoding accuracy, it doesn't provide added information about outcome prediction (Figure 8).

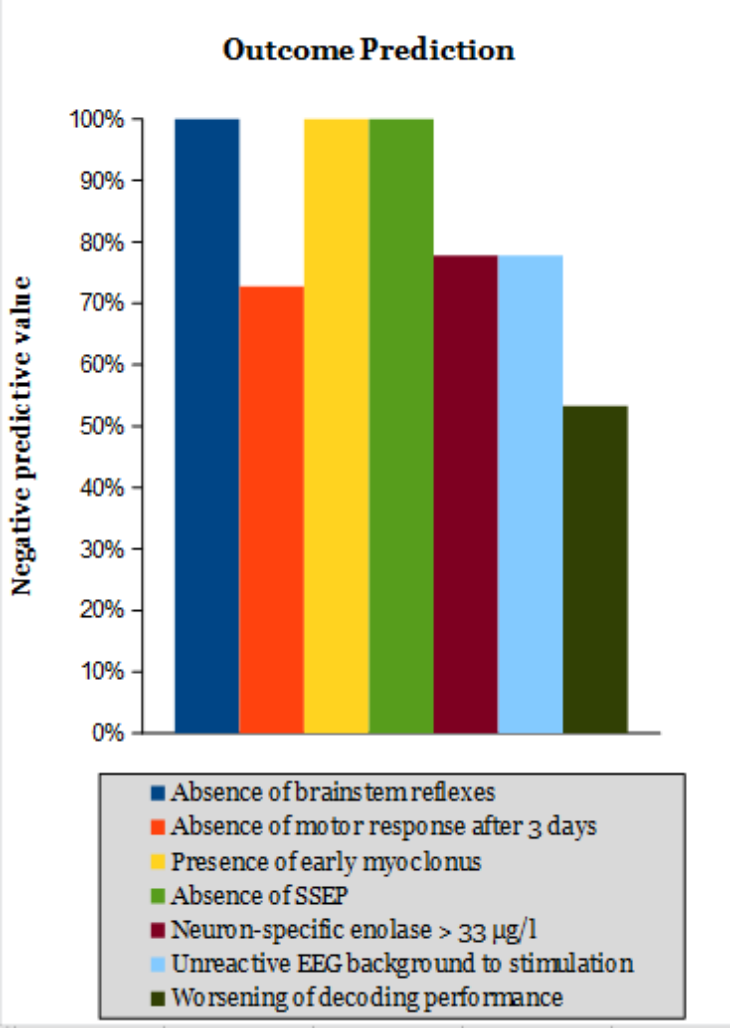

Figure 8 : Comparison of predictive value for poor outcome (NPV) of clinical and electrophysiological tests.

On the other hand, in case of improvement of decoding performance between normo- and hypothermia, all but two patients survived at three months, meaning a high PPV = 78%, (Figure 9).

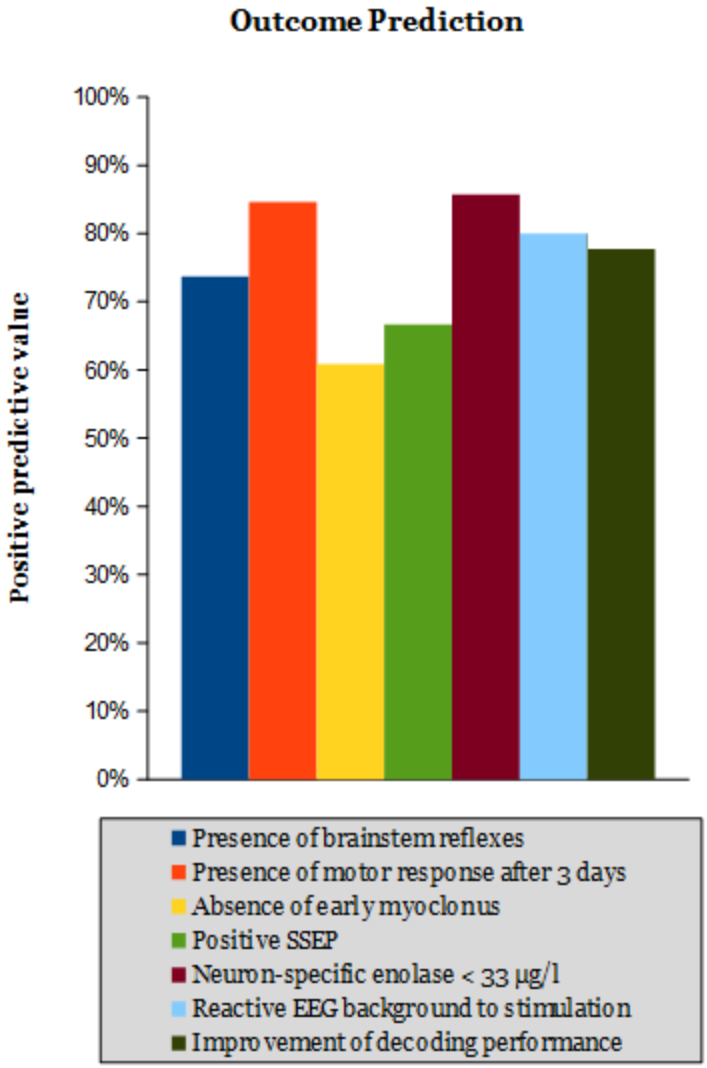

Figure 9 : Comparison of Positive predictive value (PPV) of clinical and electrophysiological tests.

In order to confirm the potential interest of our test in providing information about positive outcome in post-anoxic comatose patients, we compared decoding performance improvement with EEG reactivity, which has been demonstrated to be of interest in predicting favourable outcome among comatose patients. Indeed, in a recent study [35], 290 post-anoxic comatose patients' EEG were recorded. Reactivity to background stimulation was present in 90 patients during TH, and among them, 77 (86%) survived. In our study, 15 patients over 24 showed EEG reactivity, and 12 from them survived (PPV = 80%).

Finally, 2 patients with poor outcome despite improvement of decoding accuracy showed reactive EEG background to stimulation. We observed that NSE was higher (88.7 and 88.2 µg/l, respectively) in those two patients with a false positive test.

5. Discussion

Summary

In the present study, we found evidence supporting two main hypotheses: First, we show that a violation in a global auditory regularity as measured by EEG can be detected in patients during early coma, even during therapeutic hypothermia and under sedation. Second, an improvement of discrimination performance between hypothermia and normothermia recordings was predictive of patients' chance of surviving.

Global and local regularities

We focused on the neural correlates of auditory regularities established after repeating series of sounds (global regularity). Our results complement previous findings obtained using EEG and classical MMN paradigms in the same type of patients [17]. Indeed, we found a similar relation between auditory discrimination improvement between the two recordings and patients' 3-months survival, such as to say that a progress in auditory performance was only observed in survivors. In addition, the auditory discrimination in the present protocol was significant even in patients who will not survive similarly to what already shown in Tzovara et al., [17]. It is worth to note that another recent study [37] showed evidence of semantic categorization between different sounds of living or man-made objects in the same type of patients, but did not provide any relation between performance and outcome. All these results suggest that the progression of auditory discrimination per se is not always predictive of patients' outcome. By contrast, the present and previous studies on the progression of auditory discrimination in MMN-based paradigms are both indicative of patients' chance of survival irrespective of whether the regularities are established at the level of the single sound or at the level of groups of sounds [17].

Global discrimination in coma

In the present study, auditory discrimination performance was similar in patients with a favourable outcome and those who do not survive, both under therapeutic hypothermia and normothermia (Figure 7). We show that a violation in an auditory global regularity could be detected during early coma and under therapeutic hypothermia. This is not in accordance with several previous studies that considered detection of a global violation as a marker of conscious processing [32-33] . Those studies showed that patients erroneously labelled as vegetative or minimally conscious state could detect global auditory irregularities. By contrast, we found evidence for global violation detection among comatose patients (assessed by the Glasgow Coma Scale).

This important difference could be explained by several hypotheses: first of all, EEGs were recorded early after coma onset (after 24 and 48 hours for hypo- and normothermia, respectively), i.e. earlier and at more fixed latencies comparing to previous studies. Thus, to the best of our knowledge, this is one of the first studies to assess systematically the degree of global discrimination during acute coma. Moreover, hypothermia has been proved to provide neuroprotection, through multiple mechanisms : diminution of oxygen consumption with limitation of free radicals production, protection of hypoxia, and diminution of toxic neuroexcitation [28,29]. This could provide a possible explanation to the preservation of sound discrimination during early coma, independently of outcome.

Healthy controls

Regarding active controls, we found results in accordance with other recent studies [15,32,33], suggesting that the auditory discrimination between global standard and global deviant is improved when healthy subjects accurately count the number of deviants. As such, global auditory discrimination seems to reflect a high-level behavioural performance. In the passive condition, we found evidence of accurate discrimination in one subject, suggesting that detection of violation of auditory regularities in healthy controls can be detected even in absence of attention.

Relation with existing clinical tests

Usually, in a clinical routine, multiple criteria are used to predict outcome in post-anoxic comatose patients. Specifically, those are based on AAN guidelines [7]. They have a strong predictive value for death [2]. However, there is no reliable paraclinical tool for the moment to predict the chance of survival. Since therapeutic hypothermia implementation, EEG background reactivity has been used increasingly, because it could be an interesting tool for positive outcome prediction, with a PPV of 87% when background reactivity is observed [35]. In our study, we observed a similar PPV (78%). Thus, our auditory paradigm could offer a way to identify the patients who will survive and awaken, using a quantitative protocol, early after coma onset (within 48h). Intriguingly, we found that the patients that were misclassified based on the progression of auditory discrimination in our study had a high NSE. This phenomenon is similar to what reported by Tsetsou et al. [35] where high serum NSE was associated with mortality among similar patients despite EEG background reactivity during therapeutic hypothermia.

Limitations

Specific guidelines [2], patient's general condition, and family wishes where taken into account when deciding to withhold or withdraw life sustaining treatments. As such, a comfort care attitude was decided in 9 patients over 24, which could be a limitation in the interpretation of our results. In fact, intensive medical care was withdrawn in the two patients with a false positive test. Therefore, the potential long-term outcome of 9 patients (including our 2 patients with a false positive test i.e. dying despite improvement of sound discrimination) is not known. This could be a limitation for survival assessment of our patients. Actually, when excluding these 9 patients with intensive care withdrawal, our test shows a positive predictive value of 100%, meaning that all patients with improvement of decoding accuracy between hypo- and normothermia show long-term survival. Finally, we limited our study group to post-anoxic comatose patients in order to get a more homogeneous population, and further studies are needed to evaluate this tool in other groups of patients.

6. Conclusion

Outcome prediction in patients unable to communicate remains a major medical challenge. Clinical and standard paraclinical evaluation is not sufficient, and calls for complementary tools.

We implemented a new protocol in order to evaluate sound discrimination in noncommunicating patients and establish markers of chances of awakening. Global auditory violations could be detected during early coma, even during therapeutic hypothermia and under sedation. Moreover, an improvement of a global discrimination between hypo- and normothermia was highly predictive of patients' favorable outcome.

This protocol could provide to the physicians an early paraclinical test to predict good outcome in post-anoxic comatose patients, meaning less invasive procedures, better sharing of resources, and a quantitative tool to strengthen the hope of comatose patients' family.The present study shows that not only the classical MMN and its improvement over days is predictive of chance of awakening [17] but also auditory regularities violation detection in general.

7. Ethics

The study was approved by the Ethics Committee of the Centre Hospitalier Universitaire Vaudois (CHUV-Lausanne University Hospital), Lausanne, Switzerland.

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